FACULDADE DE MEDICINA
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Reversão da remodelagem ventricular na miocardiopatia dilatada idiopática

Ventricular reverse remodeling in idiopathic dilated cardiomyopathy

Sandra Maria Resende Amorim

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2. Amorim S, Rodrigues J, Campelo M, Moura B, Martins E, Macedo F, Silva-Cardoso J, Maciel MJ. **Left ventricular reverse remodeling in dilated cardiomyopathy - maintained subclinical myocardial systolic and diastolic dysfunction**. Int J Cardiovasc Imaging. 2017 May; 33(5), 605-613.
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3. Amorim S, Campelo M, Moura B, Martins E, Rodrigues J, Barroso I, Faria M, Guimarães T, Macedo F, Silva-Cardoso J, Maciel MJ. **Role of biomarkers in dilated cardiomyopathy- evaluation of clinical severity and reverse remodelling.** Rev Port Cardiol. 2017;36(10):709-716.
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5. Amorim S, Garcia R, Pinho T, Rodrigues J, Macedo F, Silva-Cardoso J, Maciel MJ. **Left ventricular mechanical reverse remodeling not followed by electrical reverse remodeling.** Cardiology. 2017 Jun 15;138(2):80-86.
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LIST OF ACRONYMS

ACEI= Angiotensin-converting enzyme inhibitors
ARB= Angiotensin II receptor blockers
BNP = Brain natriuretic peptide
BSA = Body surface area
CA 125= Carbohydrate antigen 125
CPET= Cardiopulmonary exercise testing
DCM = Dilated cardiomyopathy
ECM = Extracellular matrix
EF = Ejection fraction
GDF-15= Growth Differentiation Factor 15
HF = Heart failure
HFrEF = Heart failure with reduded ejetion fration
HFpEF= Heart failure with preserved ejection fration
hsCRP= high sensivity C-reactive protein
LA = Left Atrium
LAVi= Left atrial volume/BSA
LV = Left ventricle
LVDD= Left ventricle diastolic diameter
LVDDi= Left ventricle diastolic diameter/BSA
LVDVi= Left ventricle diastolic volume/BSA
LVEF= Left ventricular ejection fraction
LVM = Left ventricular mass
LVRR= Left ventricular reverse remodelling
MRI= Magnetic resonance imaging
MI= Myocardial infarction
MMP = Matrix metalloproteinase
NYHA = New York Heart Association
ST2= Soluble receptor for interleukin 33
TIMP = Tissue inhibitor of MMP
VE= Expired ventilatory flow
VO2= Oxygen uptake
VCO2= Carbon dioxide output

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RESUMO

Introdução

O processo de remodelagem cardíaca é influenciado pela sobrecarga hemodinâmica, ativação neuro-hormonal e outros fatores ainda não totalmente esclarecidos. O miócito é a principal célula envolvida, mas outros componentes da remodelagem são: o interstício, os fibroblastos, o colagénio e vascularização coronária. Além disso, outros processos importantes são: a isquemia, a necrose celular e a apoptose. Quando a remodelagem ventricular está avançada, é autossustentável e conduz à progressão da doença, independentemente do estado neuro-hormonal. Este facto explica porque é que as terapêuticas medicamentosas perdem a sua eficácia na insuficiência cardíaca (IC) terminal e algumas terapêuticas baseadas em dispositivos (como a ressincronização cardíaca ou a assistência ventricular mecânica) têm sido benéficas. A reversão da remodelagem ventricular como determinante do prognóstico não está definida e os seus mecanismos moleculares não foram totalmente investigados.

A remodelagem reversa do ventrículo esquerdo (RRVE) é caracterizada pela diminuição das dimensões do ventrículo esquerdo, normalização da forma do ventrículo esquerdo e melhoria da fração de ejeção (FEVE). Existem relatos de uma prevalência significativa de recuperação da função ventricular esquerda em pacientes com miocardiopatia dilatada (MCD). Contudo, estes estudos incluíram doentes com MCD recente, como miocardite aguda, ou com outras causas reversíveis de MCD, como a MCD periparto ou associada ao abuso de álcool. Os mecanismos subjacentes da RRVE nestas situações parecem ser diferentes dos envolvidos na MCD crónica idiopática. Os seus fatores preditores poderão discriminar os pacientes nos quais a FEVE pode recuperar com a terapêutica médica, daqueles que podem necessitar de estratégias mais agressivas. Nos pacientes com recuperação da função ventricular esquerda não está indicada a terapêutica com cardio-desfibrilhador implantado (CDI) ou ressincronização cardíaca (CRT), questionando-se o tempo de implantação destes dispositivos. Também existe um insuficiente estudo sobre os parâmetros ecocardiográficos detalhados da função miocárdica nos pacientes com FEVE normalizada após terapêutica farmacológica otimizada.

Os biomarcadores da remodelagem da matriz extracelular, de apoptose e de inflamação emergiram como potenciais indicadores na identificação de indivíduos com maior risco. Uma vez que a remodelagem do VE parece envolver vias fisiopatológicas diferentes, um painel com múltiplos marcadores poderá melhorar a estratificação de risco.

Objetivos

O principal objetivo deste trabalho foi o estudo prospetivo da recuperação da função ventricular esquerda ou reversão da remodelagem ventricular esquerda nos pacientes com MCD idiopática crónica, após a terapêutica medicamentosa otimizada. Procurou-se determinar a sua prevalência, encontrar os seus fatores preditores e verificar se esta se associou a um melhor prognóstico. Pretendeu-se também estudar de forma aprofundada os mecanismos ecocardiográficos de remodelagem reversa.

Outro objetivo foi o estudo detalhado de biomarcadores acessíveis na prática clínica e de biomarcadores experimentais. Procurou-se encontrar associações entre estes, a RRVE e parâmetros de gravidade clínica.

Métodos

Foram selecionados para o estudo 113 pacientes adultos consecutivos com MCD idiopática (diâmetro diastólico ventricular esquerdo (LVDD) > 33 mm/m2 em homens ou >32 mm/m2 em mulheres), seguidos na Consulta de Insuficiência Cardíaca do Serviço de Cardiologia do Centro Hospitalar São João, com diagnóstico estabelecido há menos de 2-3 anos e com dois valores iniciais de FEVE<0,40, com mais de um ano de separação. Foram excluídos os pacientes com MCD de etiologia secundária. A RRVE foi definida como um aumento de 10 unidades da FEVE e diminuição do LVDD na ausência de CRT ou de assistência ventricular mecânica. Realizaram-se ecocardiogramas transtorácicos seriados, análises sanguíneas com biomarcadores, prova de exercício cardiopulmonar (CPET) e ressonância magnética cardíaca (MRI).

Realizou-se um estudo ecocardiográfico detalhado (n=50, em ritmo sinusal) de análise morfológica, de função sistólica e diastólica, com quantificação da *performance* miocárdica (índices de *Tei* do VE e VD) e o estudo do *strain rate* sistólico longitudinal (SSR long) e circunferencial (SSR circ).

Foram doseados os seguintes biomarcadores (n=50): adrenalina, noradrenalina, renina plasmática, aldosterona e peptídeo natriurético tipo B (BNP) (ativação neuro-hormonal), proteína C reativa de alta sensibilidade (hsCRP), antigénio CA-125 (inflamação), ácido úrico e Lp (a) (stress oxidativo), creatinina e cistatina C (função renal), 25-OH-vitamina D (remodelagem extracelular). Também avaliámos o perfil de biomarcadores experimentais, tais como: i) marcadores de remodelagem da matriz extracelular (ECM)- metaloproteinase da matriz 3 (MMP-3), inibidor tecidular da metaloproteinase-2 de matriz (TIMP-2), proteína ST2 (receptor de interleucina 33) e Galectina-3; iii) marcadores de inflamação - receptor TNF I/TNFRSF1A (sTNF-R1); e iv) factor de diferenciação de crescimento 15 (GDF-15), um marcador de apoptose.

Resultados

A RRVE ocorreu em 34,5% dos pacientes numa mediana de 22,6 meses de seguimento. Os fatores preditores de RRVE (na análise univariada) foram: hipertensão arterial de grau ligeiro, fibrilhação auricular, hipertrofia ventricular esquerda no ECG, ausência de bloqueio completo de ramo esquerdo, menor duração do intervalo QRS, maior valor de hematócrito, menor LVDD indexado à superfície corporal (LVDDi), maior eficiência de oxigênio no pico de exercício (VO2/Log10(VE)) e menor declive VE/VCO2/VO2, tratamento com IECA/ARB e uso de doses máximas de IECA /ARB e bloqueadores-β. A análise multivariada mostrou que o uso de doses máximas de IECA/ARB foi preditor independente de RRVE. A presença de realce tardio (não isquémico) na MRI cardíaca não foi um preditor de RRVE. Os pacientes com RRVE apresentaram melhor capacidade funcional da NYHA (classe I) e menor

valor do BNP em comparação com os pacientes que não tiveram RRVE. Esta esteve associada a menor número de hospitalizações por IC, morte cardíaca ou transplante urgente.

Nos pacientes com RRVE houve uma diminuição significativa no LVDDi, diâmetro sistólico do VE, volumes diastólico e sistólico do VE, massa do VE e um aumento do índice de esfericidade. Entretanto, as medidas da função diastólica (LAVi, velocidade e', E/e'), índices de Tei do VE não foram significativamente diferentes dos valores basais. Adicionalmente, os valores finais do SSR circular e do SSR long não foram diferentes dos níveis basais. Os pacientes que recuperaram a FEVE >50%, o SSR circ e SSR long foram inferiores aos valores normais.

Não se encontrou nenhuma associação entre o BNP, 25-OH-vit D, CA 125, hsCRP, Lp (a), noradrenalina, adrenalina, renina, aldosterona e a RRVE; contudo, os pacientes em classe mais avançada de NYHA (III-IV), com congestão pulmonar ou edema apresentaram níveis mais elevados de CA 125, cistatina C, BNP e hsCRP. Os doentes com RRVE apresentaram valores basais mais baixos de Galectina-3. O BNP correlacionou-se diretamente com LVDD, volume diastólico do VE, PSAP e E/e'; foi inversamente correlacionado com a FEVE e a velocidade da onda e´. O CA 125 correlacionou-se positivamente com o volume da aurícula esquerda/superfície corporal (LAVi), relação E/A e PSAP (r=0,49). O valor da MMP-3 aumentou significativamente, ao longo do seguimento, na população total, tanto nos pacientes com ou sem RRVE. O GDF-15 aumentou (marginalmente) nos doentes que recuperaram a FEVE. O GDF-15 correlaciou-se com E/e '; o TIMP-2 correlacionou-se com LVDVi, LAVi (r = 0,28, p = 0,02) e com o índice de esfericidade.

Conclusões

A RRVE ocorreu em cerca de um terço dos pacientes com MCD idiopática, mais frequentemente naqueles com perfil tensional mais elevado e com doença menos avançada, que poderão ter beneficiado da titulação máxima dos fármacos.

Nos pacientes com LVRR houve melhoria dos volumes do VE e do índice de esfericidade, confirmando de forma "verdadeira" a remodelagem reversa do VE. Apesar da recuperação da FEVE para "valores normais", persistiram valores anormais de *strain rate* sistólico circunferencial e longitudinal, e valores anormais da função diastólica e do índice de Tei do VE, sugerindo disfunção subclínica persistente.

O CA125, BNP, hsCRP foram preditores de gravidade clínica, de congestão pulmonar e sistémica. Os valores basais mais baixos de galectina-3 poderão ser um preditor de RRVE e postula-se que a galectina-3 pode ter um impacto negativo na função cardíaca. Na análise dos níveis basais e finais de biomarcadores, houve uma queda significativa no BNP; mas alguns biomarcadores, particularmente GDF-15 e MMP-3, continuaram a aumentar, apesar da terapêutica da HF, mesmo em pacientes com LVRR. Podemos especular que, apesar da remodelagem reversa, há ativação persistente da ECM, apoptose e atividade inflamatória. Foram encontradas correlações significativas entre os níveis de biomarcadores e os parâmetros ecocardiográficos da remodelagem do VE, apoiando a via multidirecional de remodelagem na IC.

O relato de um caso clínico de recuperação da FEVE acompanhada de um evento arrítmico major, aponta para a hipótese de a remodelagem reversa mecânica do VE não ser sinónimo de remodelagem reversa elétrica, persistindo o risco arrítmico. Recomenda-se assim a continuação da terapêutica medicamentosa e manutenção da terapêutica com dispositivos cardíacos de prevenção de morte arrítmica, nos pacientes com FEVE recuperada.

Múltiplas linhas de evidência sustentam que, na maioria dos casos, a remodelagem reversa não conduz a um coração normal, apesar da reversão de muitos aspetos do fenótipo da IC. Assim, a regressão do fenótipo de IC e a recuperação no sentido de uma forma e de uma função ventricular sistólica normalizada não significa que a biologia celular/molecular e a fisiologia desses corações sejam normais. Isso poderá explicar porque é que a remodelagem reversa pode ser acompanhada por recorrência de dilatação e disfunção do VE, com resultados clínicos desfavoráveis.

Estes achados têm implicações futuras no desenvolvimento de terapêuticas que reparem de forma completa o miocárdio insuficiente. O reconhecimento deste novo fenótipo clínico, conhecido como um estado de remissão da IC, ressalta a necessidade de definir e identificar com precisão o miocárdio com remodelagem reversa para o estabelecimento da terapêutica mais apropriada.

ABSTRACT

Introduction

The process of cardiac remodeling is influenced by hemodynamic load, neurohumoral activation, and other factors that are not well understood. The myocyte is the major cardiac cell involved in remodeling, but other components include the interstitium, fibroblasts, collagen and coronary vasculature. Also, important processes are ischemia, cell necrosis and apoptosis. When ventricular remodeling is advanced, it is self-supporting and conducts to progression of the disease, independently of neurohormonal status. This explains why medical therapies lose their effectiveness in terminal HF, and some device-based therapies (like cardiac resynchronization or mechanical ventricular assistance) have been beneficial. The significance of ventricular remodeling as a prognostic determinant is not defined and molecular mechanisms of reverse remodeling have not been fully investigated.

Left ventricular reverse remodeling (LVRR) is defined by a decrease of left ventricular dimensions, normalization of left ventricular shape and improvement of ejection fraction (EF). A significant prevalence of recovery of left ventricular function in patients with dilated cardiomyopathy (DCM) has been reported. However, these studies included patients with new-onset DCM like acute myocarditis or other reversible causes of DCM, such as peripartum and alcohol-related DCM. Mechanisms underlying LVRR in such situations seem to be different from those involved in chronic idiopathic DCM. These variables probably discriminate patients in whom EF can recover with medical therapy only from those who may require more aggressive strategies. Patients whose LV function recovers no longer have indication for implantable cardiac defibrillator (ICD) or resynchronization therapy (CRT), thus complicating the timing of implantation of these devices.

There is also insufficient research about detailed echocardiography parameters of myocardial function in patients with normalized EF after optimal pharmacologic therapy.

Biomarkers of matrix extracellular remodelling, apoptosis and inflammation have emerged as potential indicators to identify individuals with major risk. Because LV remodeling seems to involve different pathophysiologic pathways, a multimarker panel may improve risk stratification.

Aims

The main aim of this prospective study was to evaluate the recovery of left ventricular function or reversal of ventricular remodeling in patients with chronic idiopathic DCM, after optimized medical therapy. We tried to determine its prevalence, to find its predictors and to check if it was associated with better prognosis. We also intended to assess the echocardiographic mechanisms of reverse-remodeled cardiomyopathy.

Another aim was to study current and experimental biomarkers of LVRR in the context of DCM. We tried to find associations between the biomarkers, LVRR and clinical severity.

Methods

We 113 adult consecutive patients with idiopathic DCM (left ventricular diastolic diameter (LVDD) >33 mm/m2 in men or >32 mm/m2 in women), followed in the HF Clinic of Cardiology Department of Centro Hospitalar de São João, with a diagnosis of less than 2-3 years duration and with two initial values of left ventricular ejection fraction (LVEF) of <0.40, more than one year apart. We excluded DCM patients with secondary etiologies. LVRR was defined as an increase of 10 units of LVEF and decrease of LVDD in the absence of CRT and ventricular assistance therapy. We performed serial transthoracic echocardiograms, blood laboratory measurements with current and novel biomarkers, cardiopulmonary exercise testing (CPET) and cardiac magnetic resonance imaging (MRI).

We also performed an echocardiographic study in a subgroup (n=50, in sinus rhythm) of detailed morphological, systolic and diastolic function analysis, with myocardial performance quantification (LV and RV Tei indexes) and LV averaged peak systolic longitudinal strain (SSR long) and circumferential strain (SSR circ).

In the biomarker substudy (n=50) we measured: adrenalin, noradrenalin, plasma renin, aldosterone and B-type natriuretic peptide (BNP) (neurohormonal activation), high-sensitivity C-reactive protein (hsCRP), cancer antigen CA-125 (inflammation), uric acid and Lp(a) (oxidative stress), creatinine and cystatin C (renal function), 25-OH-vitamin D (extracellular remodeling). We also evaluated the profile of emerging biomarkers, such as i) markers of ECM remodeling including matrix metalloproteinase -3 (MMP-3), tissue inhibitor of matrix metalloproteinase- 2 (TIMP-2), protein ST2 (receptor for interleukin 33) and Galectin-3; ii) markers of inflammation such as soluble TNF receptor I/TNFRSF1A (sTNF-R1); and iv) growth-differentiation factor 15 (GDF-15), a marker of apoptosis.

Results

LVRR occurred in 34.5% within 22.6 months. Univariate predictive factors of LVRR were: mild hypertension, atrial fibrillation, ventricular hypertrophy on EKG, absence of LBBB, shorter QRS duration, higher hematocrit, lower LVDD/BSA (LVDDi), higher oxygen efficiency at peak exercise (VO2/LG10 (VE)) and a lower dVE/VCO2/VO2, treatment with ACEI/ARB and use of maximal doses of ACEI/ARB and β- blockers. Multivariate regression analysis showed that the use of higher doses of ACEI/ARB was independently associated with LVRR. The analysis of non-ischemic delayed enhancement in cardiac MRI was not a predictor of LVRR. Patients with LVRR had a better NYHA functional capacity (class I) and had lower BNP compared to those without LVRR. LVRR was associated with lower rates of HF hospitalization, cardiac death or urgent transplantation.

In patients with LVRR, there was a significant decrease in: LVDDi, LV systolic diameter/BSA, LV diastolic volume, LV systolic volume, LV mass; an increase in sphericity index. However, measures of diastolic function (LAVi, e´velocity and E/e´ratio), final LV and RV Tei indexes were not significantly different from baseline. Additionally, final SSR circ and SSR long values were not different from basal. Patients who recovered EF> 50% SSR circ and SSR long were inferior to normal.

No association was found between BNP, 25-OH-vit D, CA 125, hsCRP, Lp (a), noradrenalin, adrenalin, renin, aldosterone and LVRR; however, patients in NYHA class (III-IV), with pulmonary congestion or ankle oedema had higher levels of CA 125, cystatin C, BNP and hsCRP. Patients with pharmacological LVRR had lower baseline values of galectin-3. BNP correlated directly with LVD, LV volumes, PSAP and E/e´; and was inversely correlated to LVEF and e´velocity. CA 125 positively correlated with left atrium volume/BSA (LAVi), E/A ratio and PSAP (r=0,49). MMP-3 increased in the overall population, both in patients with or without LVRR. GDF-15 correlated with E/e'; TIMP-2 correlated with LVDVi, LAVi (r=0.28, p=0.02) and sphericity index. MMP-3 had a positive correlation with LVEF. GDF-15 increased (marginally) in patients who recovered LVEF.

Conclusions

LVRR occurred in about one third of IDCM patients, especially in those with higher blood pressure and with less advanced disease, which may benefit of maximal drug titration.

In patients with LVRR there was an improvement of diastolic and systolic volumes and in sphericity index, confirming truly LV reverse reshaping. Besides recovery of left ventricular ejection fraction to "normal values", there is still abnormal circumferential and longitudinal left ventricular strain rate and left ventricular Tei index values, suggesting persistent subclinical left ventricular dysfunction.

CA125, BNP and hsCRP were predictors of clinical severity and congestion. Lower baseline values of Galectin-3 may be a predictor of LVRR in DCM patients and we postulate that galectin-3 may have negative impact on cardiac function.

In the analysis of basal and final levels of biomarkers in our population, there was a significant decrease in BNP; but some biomarkers, particularly GDF-15 and MMP-3, continued to rise, despite HF therapy, even in patients with LVRR. We can speculate that despite ventricular reverse remodeling, there is persistent matrix fibrosis activation, apoptosis and inflammatory activity. Significant correlations were found between the levels of biomarkers and echocardiographic parameters of LV remodeling, supporting the multidirectional pathway of remodeling in HF.

The report of LV recovery case with a major arrhythmic event point the hypothesis that LV mechanical reverse remodeling is not always followed by LV electrical reverse remodelling, with persistence of arrhythmic risk. Our believe is to continue background medical or device therapy for sudden death in HF-Recovered patients.

Multiple lines of evidence support the point of view that in most instances, reverse remodeling does not lead to a normal heart, despite reversal of many aspects of the HF phenotype. Thus, the regression of the HF phenotype and the accompanying return toward a more normal ventricular shape and function does not signify that the cellular/molecular biology and physiology of these hearts is normal. This may explain why reverse remodeling may be followed by recurrence of LV dilatation and dysfunction with poor clinical outcomes. These findings have implications for future research to develop therapies to repair fully the failing myocardium. Recognition of this new clinical phenotype, which is coming to be known as a

state of HF remission, underscores the need to accurately define and identify reverse modelled myocardium in order to investigate the most appropriated therapies.

CHAPTER I

INTRODUCTION

1. HEART FAILURE MECHANISMS

Heart failure (HF) is one of the most relevant causes of morbidity and mortality in the industrialized world (1). This syndrome carries a mortality of ≈50% at 5 years, and its incidence and prevalence are expanding around the globe (2). The prevalence of symptomatic HF is estimated to range from 0.4 to 2.0% in general European population (3). The lifetime risk of HF at age 55 years is 33% for men and 28% for women (4, 5). The estimated lifetime cost of HF per individual patient is $110,000/year, with more than three-fourths of this cost related to hospital care (6). Despite the improvement in therapeutics, the 5-year mortality is still approximately 50%, which is worse than many cancers (7).

HF is triggered by an initial event that can be abrupt, such an acute myocardial infarction, or insidious like: pressure or volume overload, genetic or toxic factors. The common feature of each of these events is that they all produce a decline in ventricular function (8). In Figure 1. is presented a scheme of Heart Failure Mechanisms, based from text of Mann et al.(8).

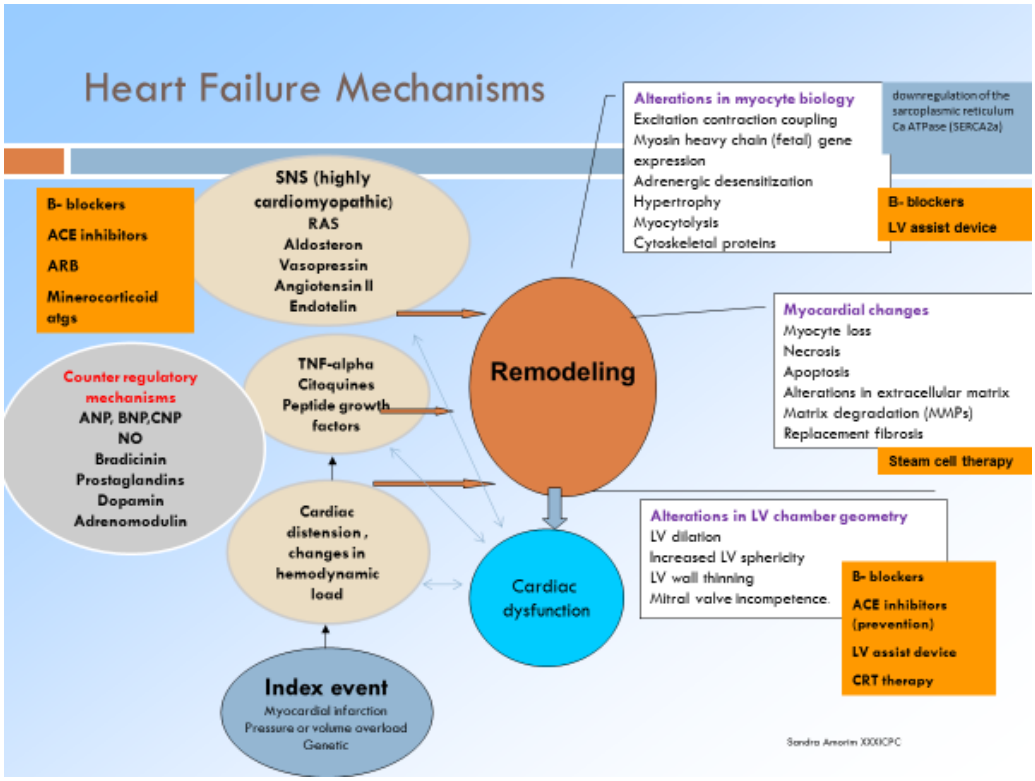

Figure 1- Heart Failure Mechanisms

Sandra Amorim- Personal communication- XXXI Portuguese Congress of Cardiology, Joint Session with the European Society of Cardiology, 2010. Adapted from text of Mann (8).

Clinicians initially viewed HF as a problem of excessive salt and water retention that was caused by abnormalities of renal blood flow (the "cardiorenal model"). It also became apparent that HF was associated with a reduced cardiac output and excessive peripheral

vasoconstriction (cardiocirculatory model) (9). The cardiorenal models provided the rational basis for the use of diuretics to control the volume status, and the cardiocirculatory model provided the rational basis for the use of inotropes and intravenous vasodilators to augment cardiac output, these therapeutic strategies have not prevented HF from progressing (9-11). However, neither of these models explain the disease progression.

1.1 Neurohormonal compensatory mechanisms

Compensatory mechanisms are activated after the initial decline in the cardiac function, those include activation of the adrenergic nervous system and salt- and water-retaining systems in order to maintain cardiac output (8, 12, 13). Simultaneously there is activation of vasodilatory molecules, to counteract the excessive vasoconstriction resulting from excessive activation of the adrenergic and renin-angiotensin systems (14). Norepinephrine, angiotensin II, endothelin, aldosterone, and TNF are active molecules, sufficient to contribute to disease progression in the failing heart; vasodilators such as nitric oxide, natriuretic peptides, prostaglandins, and kinins, are insufficient to counteract the peripheral vasoconstriction. The increase in cardiac adrenergic drive through β1-adrenergic receptor pathways is the most powerful compensatory mechanism, with an increase in contractile function, in heart rate, myocyte hypertrophy, volume expansion and an increase in left ventricular tele diastolic volume (by signalling vasopressin release)(8, 15).

It may not be possible to achieve complete inhibition of the renin-angiotensin system or the adrenergic system in HF because of dose-limiting side effects of ACEI and β-blockers; there may be alternative metabolic signalling for neurohormones (conversion of angiotensin I to angiotensin II within the myocardium by tissue chymase); biologically active molecules may contribute to disease progression due to toxic effects on the heart and the circulation; some HF activated signalling pathways that produces harmful effects in cardiac myocytes in isolated systems (like endothelin or TNF-α) may have favourable effects in the complex HF scenario (8). Finally, HF can progress independently of the neurohormonal status of the patient.

1.2 Ventricular Remodeling

Cardiac remodeling is due to an abnormal genomic expression that translates into molecular, cellular and interstitial changes leading to changes in the size, shape and function of the heart, in response to aggression or hemodynamic overload of the heart (16). Ventricular remodeling is, therefore, an important aspect in the progression of HF, additional of the neurohormonal state. This process is the culmination of a complex series of transcriptional, signalling, structural, electrophysiological, and functional events occurring within the cardiac myocyte (17).

Although the term "cardiac remodeling" was initially named to describe the prominent changes that occur following myocardial infarction (18, 19) similar processes occur following other types of injury such as with pressure overload (aortic valve stenosis, hypertension),

inflammatory disease (myocarditis), idiopathic dilated cardiomyopathy (DCM), and volume overload (valvular regurgitation).

Pfeffer et al. introduced the fundamental concept that the structural aspects of remodelling are defined and quantified by shifts of the ventricular end-diastolic pressure–volume relationship (EDPVR) a finding that was soon confirmed in human end-stage failing hearts obtained at the time of heart transplantation (20).

Different forms of myocardial aggression share molecular, biochemical and cellular events leading to change in the shape and function of the myocardium (8).

1.2.1 Myocyte hyperplasia and hypertrophy

Most cardiomyocytes are incapable of dividing (hyperplasia) and respond to stress with a hypertrophic growth response. As part of this process, a wide range of transcriptional and posttranslational events occur, including activation of a pattern of gene expression reminiscent of that observed during foetal development (foetal gene program)(2).

Remodeling is associated with myocyte hypertrophy following increased production and local release of angiotensin II, noradrenaline and endothelin. Load changes cause cytoplasmic membrane distension and increase wall stress, which also induces the expression of genes associated with hypertrophy. The stretching of the myocyte membrane further increases the release of AII, with local increase of ACE and expression of AT1 receptors. The increase in angiotensin II, aldosterone and cytokines stimulate the synthesis of collagen, causing fibrosis and remodeling of the extracellular matrix (ECM) (21). Increased wall stress may precipitate an imbalance between energy intake and energy consumption, leading to ischemia and to a vicious cycle of functional aggravation (17).

1.2.2 Alterations in myocyte biology

The changes that occur in the biology of the failing adult cardiac myocyte include: 1) cell hypertrophy; 2) changes in excitation-contraction coupling leading to alterations in the contractile properties of the myocyte; 3) progressive loss of myofilaments (myocytolysis); 4) β-adrenergic desensitization; 5) abnormal myocardial energetics secondary to mitochondrial abnormalities and altered substrate metabolism; and 6) progressive loss and/or disarray of the cytoskeleton (22).

In myocyte biology, there is a decrease in the expression of the α-myosin heavy chain gene with increased expression of β-myosin heavy chain, a progressive loss of myofilaments, changes in cytoskeletal proteins, downregulation of expression of calcium ATPase of sarcoplasmic reticulum (SERCA2a), changes in the contraction-excitation coupling and desensitization of β-adrenergic receptors. This is accompanied by the activation of a program of foetal cardiac genes that includes genes whose products regulate cardiac contractility and calcium metabolism. There is dysregulation of Ca2+ movements: a diastolic leak of Ca2+ through altered RyR2 lowers the Ca2+ content of the sarcoplasmic reticulum, reducing the Ca2+ that can be released during activation, thereby weakening contraction (23). Another

major abnormality of Ca2+ fluxes is the loss of function of the SERCA2a pump, which reduces the Ca2+ content of the cardiac sarcoplasmic reticulum, and the quantity the Ca2+ released during myocyte activation, causing systolic dysfunction and ventricular tachyarrhythmias (24).

Additionally, stimulation of β-adrenergic receptors normally causes the phosphorylation of phospholamban and thereby disinhibits SERCA2a, enhancing both cardiac contraction and relaxation; this "contractile reserve" provided by adrenergic stimulation may be reduced in HF, with the desensitization of myocardial β-receptors (25).

1.2.3 Myocyte death and apoptosis

Myocyte necrosis may be caused by norepinephrine, endothelin, and circulating AII in the myocardial tissue. The occurrence of myocyte necrosis is suggested by studies showing that troponin I circulation were increased 3 to 4 times in patients with advanced heart failure (26).

Myocardial apoptosis (programmed cell death) can be triggered by myocardial stretch, norepinephrine, oxidative stress, TNF-α, and angiotensin II (27, 28). As angiotensin II and norepinephrine can trigger apoptosis, drugs that block sympathetic nervous system and renin-aldosterone system can reduce apoptosis (29, 30).

In autophagy, cells digest their own intracellular proteins and lipids, a process that may be normal (protective) when these substances are altered and become toxic, but when accelerated may become maladaptive and result in increased cell death (31). Selective inhibitors targeting apoptosis (caspase inhibitors), necrosis (inhibitors of mitochondrial permeability transition pore opening), and necroptosis (necrostatin1) have been developed (32). However, optimal timing of therapy, targets for inhibition within apoptotic signalling cascades, precise mechanisms of inhibition, and even the cell types involved remain unclear (2).

1.2.4 Fibrosis

The cardiac ECM is a dynamic structure of a complex network of fires comprised of matrix proteins in which cardiac myocytes, leucocytes, cardiac vascular cells, and fibroblasts are involved. In the ECM, perivascular fibrosis occurs, as well as replacement fibrosis (8). The increased synthesis of ECM enhances myocardial stiffness in pressure overload hypertrophy and reduces the rate of ventricular relaxation as well as contraction (33). This promotes contractile dysfunction and rhythm disturbances. In myocardial infarction scar formation arises from replacement fibrosis in regions of myocyte necrosis; in hypertension, in pressure overload and in remote regions after myocardial infarction, fibrosis is reactive (perivascular or interstitial)(2). Experimental studies have shown that angiotensin II, endothelin and aldosterone can cause excessive fibrosis in myocardial tissue (34, 35).

Activation of matrix metalloproteinases (MMPs) (collagenolytic enzymes) leads to a progressive degradation of the ECM, which in turn leads to mural realignment ("slippage") of myocytes in the left ventricle, which contributes to ventricular dilatation and decreases the thickness of the wall (8). TNF-α, cytokines and peptide growth factors can activate MMPs. Degradation of the matrix is also controlled by inhibitors of matrix metalloproteinase

glycoproteins (TIMPs). Myocardial fibrosis is associated with ECM degradation resulting from an imbalance between MMPs and tissue inhibitors of MMPs, favouring the latter, and causing excessive fibrosis (36).

One study showed that anti-angiotensin and anti-mineralocorticoid intervention leads to an increase in scar maturation while diminishing remote reactive fibrosis (37). Angiotensin receptor blockers (ARB) appear to have antifibrotic actions; losartan reduced fibrosis and serum collagen markers in hypertensive heart disease (38, 39). There is no current clinical evidence to suggest that fibrotic changes occurring in the myocardium are completely reversible (8).

1.2.5 Vascular remodelling

The development of coronary collateral circulation is an important mechanism of vascular remodeling. Promoting angiogenesis in the setting of pressure overload can protect the heart from injury (40). Clinical trials of therapeutic neovascularization with gene or protein therapies have failed. The development of proangiogenic therapies may require a combination of multiple growth factors such as fibroblast growth factor-2, hepatocyte growth factor, monocyte chemoattractant protein-1, granulocyte macrophage colony-stimulation factor, platelet-derived growth factor-β, and transforming growth factor-β (2, 41).

1.2.6 Metabolic remodelling

There is an association between altered metabolism, insulin resistance and HF. This may be due to several causes: activation of the neurohormonal system; inflammation as exemplified by increased tumour necrosis factor α (TNF-α) levels and its soluble receptors; alterations in skeletal muscle function and mass as a result of reduced physical activity; endothelial dysfunction; increased adipokines such as adiponectin and leptin; and pharmacological exacerbation insulin resistance (e.g. by diuretics) (42).

1.2.7 Alterations in LV chamber geometry

Changes in the biology of the cardiac myocyte and in ECM lead to progressive LV dilatation and increased sphericity of the ventricle. The resultant increase in LV end-diastolic volume along with concomitant LV wall thinning, leads to functional ventricular afterload mismatch that contributes further to a decrease in stroke volume. High end-diastolic wall stress can lead to: 1) hypoperfusion of the subendocardium, with resultant ischemia and worsening of LV function; 2) increased oxidative stress with resultant activation of families of genes that are sensitive to free radical generation and 3) sustained expression of stretch-activated genes (angiotensin II, endothelin, and TNF-α) and/or stretch activation of hypertrophic pathways (22). The increased sphericity of the ventricle leads to functional regurgitation of the mitral valve. This process is associated with a continuous LV dilatation and a decline in the ejection fraction.

1.2.8 Electrophysiological remodelling

Electrical remodeling, which is characterized by alterations in multiple electrogenic transport processes within the cardiac myocyte, is an important pathophysiological mechanism in HF. Myocardial fibrosis, with altered electrotonic coupling between cells, slowed conduction, and dispersion of refractoriness, enhances the proarrhythmic phenotype. In HF, phosphorylation of the ryanodine receptor by Ca2+/calmodulin-dependent protein kinase II, results in calcium leakage from sarcoplasmic reticulum. There is a concurrent downregulated expression of the SERCA2a and a reduced Ca2+ uptake into the sarcoplasmic reticulum. Depletion of sarcoplasmic reticulum Ca2+ stores, coupled with elevations in cytoplasmic Ca2+, potentiates the development of ventricular arrhythmias (43, 44).

1.3- Biomarkers

There is substantial variation in the severity and prognosis of HF, ranging from mild disease, that is easily managed with neurohormonal blockade, to advanced illness requiring therapy with mechanical support or heart transplantation (45). Physicians have used biomarkers as additional tools to aid clinical diagnosis, treatment and better identify high-risk subjects (46). One of the most important areas of biomarker investigation is the role of biomarker profiling to better characterize the phenotype of patients who might best respond to therapeutic interventions be it drug or device therapies (47).

Available biomarkers reflect multiple biological processes, including inflammation, oxidative stress, neurohormonal activation, vascular remodeling, myocyte injury and renal impairment (36, 48, 49):

1- **Inflammation**: C-reactive protein (CRP), TNF-α, Tumor necrosis factor-like weak inducer of apoptosis, Interleukin (IL) -1, IL-6, IL-10, and IL-18, Lipoprotein- associated phospholipase, Soluble TNF receptors, Chitinase-3-like protein, Interleukin-1 receptor antagonist, Midkine, Leucine-rich 2-glycoprotein, Pentraxin-3, CA-125, S100A8/A9 complex, Osteoprotegerin, Serine protease PR3, Soluble endoglin, Adiponectin.

2- **Oxidative stress**: Oxidized low-density lipoproteins, Myeloperoxidase, Urinary biopyrrins, Urinary and plasma isoprostanes, Urinary 8-hydroxy-20-deoxyguanosine, Plasma malondialdehyde.

3- **Extracellular matrix remodeling:** MMPs, TIMPs**,** IL-6**,** Collagen propeptides**,** N-terminal collagen type III peptide**,** Myostatin**,** Syndecan-4**,** Galectin-3, Soluble receptor for interleukin 33 (ST2).

4- **Neurohormones:** Norepinephrine, Renin, Angiotensin II, Aldosterone, Arginine vasopressin/C-terminal pro-arginine vasopressin (Copeptin), Endothelin-1, Urocortin, Chromogranin A and B, Adrenomedullin/mid-regional pro-adrenomedullin.

5- **Myocyte injury and apoptosis:** Troponins I and T, Myosin light-chain kinase I, Heart-type fatty-acid binding protein, Creatine kinase-MB fraction, sFAS, FAS ligand, Heat shock protein-60, sTRAIL, Growth differentiation factor-15 (GDF-15).

6- **Myocyte stress:** BNP, NT-proBNP, MR-proANP, proBNP, ST2.

7- **Extracardiac involvement**: Red blood cell distribution width, Renal function and injury markers, β2-microglobulin, Urinary albumin-to-creatinine ratio, Triiodothyronine.

Current guidelines recommend testing BNP or NT-proBNP (50). In study of ambulatory patients with chronic HF, Ky et al. (51) tested in ambulatory patients with chronic HF, the hypothesis that a group of 7 biomarkers (BNP, Soluble fms-like tyrosine kinase receptor, hsCRP, ST2, cTnI, Uric acid, Creatinine), could be combined into a multimarker score that would predict the risk for an adverse outcome, defined as death, cardiac transplantation or placement of a ventricular-assist device. Patients in the highest tertile of the multimarker score had a 13.7-fold increased risk of adverse outcomes compared with the lowest tertile. Shah et al (52) examined an array of biomarkers in 21 patients suffering from refractory cardiogenic shock receiving mechanical circulatory support with a percutaneous ventricular assist device. The investigators measured a panel of biomarkers reflecting multiple pathophysiologic processes (BNP, hsCRP, soluble tumor necrosis factor receptor-1 (sTNFR1), soluble Fas, soluble Fas ligand (sFasL), endothelin-1, and procollagen III N-terminal peptide (PIIINP)), both before and after percutaneous ventricular assist device support; ventricular unloading and restoration of circulation were associated with reductions in serum BNP, sFas, and endothelin-1 levels and increases in serum sFasL and PIIINP levels.

Data now suggest that biomarkers may also be useful to predict or monitor left ventricular reverse remodeling (LVRR). A new clinical score that includes a remodeling biomarker, the ST2-R2 score, which contains five clinical variables (non-ischemic etiology, absence of LBBB, HF duration, LVEF and β-blocker treatment) and ST2. A significant relationship was observed between ST2-R2 scores and changes in left ventricular ejection fraction (LVEF) and indexed LV sizes, percentage reduction in LV end-systolic volume index, a similar trend was observed with diastolic parameters (53).

2- LEFT VENTRICULAR REVERSE REMODELING

2.1 Spontaneous left ventricular reverse remodeling (LVRR)

Spontaneous improvement in LVEF has long been recognized, even in the absence disease specific therapies. LVRR may occur spontaneously, most commonly in previously healthy young patients with a recent onset of symptoms, usually within 3 to 6 months of diagnosis (54, 55). Those patients have a higher potential of LVRR, either due to resolved underlying disease, as in the case of myocarditis; or due to the favorable effects of therapy. Kubanek and colleagues (56) reported a prevalence of 45% of LVRR at 12 months in 44 patients with recent-onset DCM that included some patients with active and resolving myocarditis. Most reports of reversible cardiomyopathy are associated with treatment of specific causes, such as tachycardia (57) endocrine disorders, sepsis (58, 59) and myocarditis (54, 60) or with cessation or moderation of alcohol (61).

In one study, 36% of subjects with HF-Recovered LVEF did not have a documented reversible cause, highlighting that recovery of LV function is also common in patients with idiopathic cardiomyopathy (62). It is unclear whether such cases of LV recovery in the absence of myocarditis, tachycardia, or toxic injury reflect an unidentified transient injury (e.g. myocarditis) or is the success of medical treatment.

2.2 The new entity- Heart Failure with Recovered ejection fraction

Consistent with the importance of remodeling in HF is the observation that some medical therapies and cardiac devices that increase the survival of patients with HF may delay and, in some cases, even revert certain parameters of remodeling. A favorable response to drug therapy in clinical trials with ACEI, β-blockers and aldosterone antagonists was reported, with almost complete reversal of left ventricular function (63-67). These agents have dramatically changed the prognosis in HF and the beneficial effects are due to favourable impact on the biology of the myocyte. An increase in LVEF of more than 15 units has been described, associated with an increase in functional capacity, an increase in cardiac index and a decrease in pulmonary capillary pressure, associated with a better prognosis (65-68).

Reversal of remodeling is also described with cardiac resynchronization therapy (CRT) (69-71), use of mechanical ventricular assist devices (LVADs) (72), stem cell and myoblasts transplantation (73).

Studies of human hearts explanted from transplantation recipients after a period of mechanical circulatory support with LVADs showed for the first time that the end-diastolic pressure-volume relationship (EDPVR) of human end-stage failing hearts can substantially shift back to normal values. Such studies allowed for definitive differentiation between LV size reduction owing to decreased preload along a fixed EDPVR (that is, unloading) and shifts of the EDPVR back to normal values. The observations from this study led us to introduce the term "reverse remodelling" (74).

HF with reduced ejection fraction (HfrEF) and HF with preserved ejection fraction (HFpEF) have different pathophysiology and treatment. According to LW Stevenson it may be

appropriate to classify the population that experiences LVRR as HF-better-EF (75). This population of HF with a low EF that improves has been recognized as a distinct population in the latest American College of Cardiology Foundation/American Heart Association Guidelines, where it has been recommended that further investigation be devoted to optimizing therapies for this new diagnosis (76). Collaborative clinical and molecular study of this modern population will provide new insight into fundamental questions of how HF compensates and what can truly recover, and ultimately help us to support and sustain recovery for patients with HF (75).

Contractile reserve has been suggested as a key predictor of LVRR, according to studies with dobutamine echocardiography (77) and positron emission tomography (78).

2.3- Definitions of Recovered EF

Some studies defined HF with recovered EF an increase to ≥40% with ≥5% absolute change (62), other studies considered patients whose EF improved to ≥50% (79). Other authors (80, 81) defined LVRR as an LV end diastolic diameter < 55 mm and an increase fractional shortening≥ 25%. The majority of studies defined LVRR as an improvement of LV EF≥ 10% in absolute value together with a decrease in LV end diastolic dimension (≥10%) and a final value of LVEF ≥35%-40% (56, 59, 82). Choi et al (83) considered LVRR an absolute increase in LVEF ≥ 20% or ≥10% in a follow-up LVEF≥50%.

2.4- Studies with Cardiac magnetic resonance imaging

Cardiac magnetic resonance imaging (MRI) may be useful in predicting LVRR, in patients with idiopathic DCM. In one study of recent-onset DCM, the lower extent of late gadolinium enhancement (LGE) and the higher edema ratio at cardiac MRI were the most important baseline predictors of LVRR (56). Another study in recent onset-DCM, sixty-eight patients with DCM and 19 healthy volunteers were studied; strain desynchrony index and fibrosis mass were independently associated with change in the LVEF over time ($P \leq 0.001$) and LGE conferred additive value for predicting change in the LVEF beyond clinical and desynchrony parameters (85).

Seventy-five patients with newly diagnosed idiopathic DCM under optimal therapy were assessed at baseline using LGE-MRI and endomyocardial biopsy (EMB); the former measured LGE area and the latter measured collagen volume fraction (CVF) as possible predictive indices of LVRR and cardiac event-free survival. Multivariate analysis indicated that only LGE area was an independent predictor of subsequent LVRR. There was no significant difference in prognosis between patients with CVF values above (severe fibrosis) and below (mild fibrosis) the median of 4.9%. The degree of myocardial fibrosis estimated by baseline LGE-CMR imaging, but not that estimated by baseline EMB, can predict LVRR and cardiac event-free survival in response to therapy in patients with newly diagnosed idiopathic DCM (86).

3- ANTI-REMODELING THERAPIES

3.1 Pharmacological therapies

3.1.1. Inhibitors of the Renin-Angiotensin-Aldosterone Axis

Angiotensin receptor activation can induce cardiac remodeling independently of changes in blood pressure. Numerous clinical trials have demonstrated that ACEI and ARBs reduce HF morbidity and mortality (73). ACEI therapy seems to prevent the worsening of LV dilatation and hypertrophy, however, there is controversial if it can induce reverse remodeling. Captopril did not lead to a reduction in LV volume, but attenuated progressive LV dilatation, in 59 patients after anterior myocardial infarction and baseline ejection fraction<45% (18). Compared to placebo, captopril, was associated with a significantly lower LV end-systolic volume and higher LVEF after 3 months of therapy in 100 patients after myocardial infarction (87). In the echo substudy of the SOLVD trial, enalapril treatment prevented further LV enlargement, associated with a slight reduction in LV mass, over a follow-up period of 12 months (65). However, in the SAVE trial, captopril attenuated LV dilatation within the first year after myocardial infarction, but not in the second year of follow-up (88). This indicates that patients may escape from the beneficial effects of ACE inhibition on maladaptive remodeling after prolonged periods of time.

The large ValHeFT trial (n=5010) demonstrated that combination therapy of the AT1 receptor antagonist Valsartan with an ACEI was more effective than ACEI therapy alone to induce reverse remodeling in patients with symptomatic systolic HF: both ejection fraction increased, and left ventricular end-diastolic dimension (LVDD) decreased significantly more with combined renin-angiotensin-aldosterone blockade over prolonged (>24 months) periods of time (89).

The renin inhibitor Aliskiren blunts remodelling in experimentally infarcted mouse hearts (90), but the ATMOSPHERE study (aliskiren versus enalapril or combination) found no benefit from the addition of a renin inhibitor to an evidence-based dose of enalapril (91).

The EMPHASIS-HF study revealed that eplerenone reduces mortality and hospitalization in patients with systolic dysfunction and mild symptoms (92). Increased levels of cardiac aldosterone have been reported in experimental models of myocardial infarction, correlating with LV remodelling. The effects of aldosterone are like those observed with angiotensin II, including inhibition of nitric oxide synthase and promotion of inflammation, fibrosis, and cardiac myocyte apoptosis.

Angiotensin receptor–neprilysin inhibitors are a new class HF drugs. They provide a new strategy of treatment named neuro-hormonal modulation by blocking the renin-angiotension-aldosterone axis and simultaneously stimulating the counter-regulatory systems through neprilysin inhibition. A drug, LCZ696, combines a moiety of valsartan with sacubitril, a neprilysin inhibitor. In the recent PARADIGM-HF study, LCZ696 was superior to enalapril in reducing the risks of cardiovascular death and of hospitalization for HF (93).

3.1.2. β-blockers

Hall et al. (94) reported a progressive increase in LV ejection fraction, a decrease in LV dilatation and hypertrophy, and LV restoration to a more elliptical shape in patients treated with metoprolol over a time course of 3–18 months. In a substudy of the MERIT-HF trial, patients treated with metopolol CR/XL were followed by cardiac MRI and a significant decrease in LV volumes and increase in LVEF were observed after 6 months in the metoprolol group, but not in the placebo group (66). Olsen et al. (95) reported LVRR in chronic HF patients treated with carvedilol. In elderly patients with HF and advanced LV systolic dysfunction, nebivolol reduced LV size and improved LVEF as reported in the echocardiographic substudy of the SENIORS trial (96). A meta-analysis of all available β- blockers trials showed an average 29% relative increase in ejection fraction, irrespective of the etiology of HF (97). In these studies, LVRR was observed with selective or nonselective beta-blockers, even in the presence of baseline therapy with an ACEI.

The results of the REVERT (Reversal of Ventricular Remodeling with Metoprolol-LX) showed that metoprolol succinate therapy reverses LV remodelling and dysfunction in patients even with asymptomatic LV systolic dysfunction (98).

Reverse remodeling with β-blocker therapy has also been documented at a cellular level in isolated human myocardium. Some beta-blockers may increase the number of β-adrenergic receptors, which are downregulated in HF (99). Lowes et al. (100) demonstrated an association between normalization of myocardial gene expression for SERCA2a and β-myosin heavy chain and the improvement in LVEF and clinical status in patients with DCM under β-blocker therapy. Reiken et al. (101) showed that β-blocker therapy partially restored diastolic filling, β-adrenergic responsiveness and ryanodine release channel function in patients with idiopathic or ischemic cardiomyopathy.

Of major relevance is the contribution of adrenergic receptor polymorphisms on HF progression and on therapeutic response. For example, a double adrenergic receptor polymorphism, an A-2C deletion/loss of function genotype (α-2C Del322-325), combined with a high-functioning β1-receptor genotype (β1 Arg389), confers a 10-fold risk for the development of HF (102).

3.1.3. Ivabradin

Ivabradin induces LVRR in patients with HF and reduced LVEF. In a rat model of chronic mild HF, ivabradine preserved cardiac output and improved LV function and geometry and these changes were linked to modifications in the ECM and in cardiac myocyte function (103). Ivabradine also had beneficial effects on the global cardiac remodelling process, including optimization of energy consumption, reverse electrophysiological and structural cardiac remodeling (104). Similar effects with ivabradine have been found by other workers in a rat model of chronic severe HF, including reductions in fibrosis, local renin-angiotensin stimulation, endothelial dysfunction and sympathetic activation (105).

The SHIFT echocardiographic substudy included 411 patients and evaluated ivabradine effect on LV remodelling, superimposed on background therapy optimal for HF (106). Ivabradine reduced LV end-systolic volume index (LVSVi) vs. placebo. The reduction in LVSVi was independent of β-blocker use, HF aetiology, and baseline LVEF. Ivabradine also improved LV end-diastolic volume index (LVDVi) and LVEF. The incidence of the SHIFT primary composite outcome (cardiovascular mortality or hospitalization for worsening HF) was higher in patients with LVSVi above the median (59 mL/m2) at baseline and patients with the largest relative reductions in LVEVi had the lowest event rates. Finally, there was a significant inverse relationship between the change in heart rate and the change in LVEF, which means that larger decreases in heart rate during follow-up were associated with greater increases in LVEF.

Ivabradin induced reversal of myocardial fibrosis and improvement of deranged E-C coupling in rats, superior to metoprolol (107).

3.1.4. Novel pharmacological therapies for LV remodeling

- **Soluble tumour necrosis factor-a (TNF-a) antagonist etanercept**

Treatment with the soluble tumour necrosis factor-a (TNF-a) antagonist etanercept improved LV remodelling and dysfunction in a study of patients with severe HF (108), however it was not associated with an improvement in clinical outcome in larger studies (109). The reasons for this discrepancy are not completely understood, but may include potential adverse effects of TNF-a antagonism and potential beneficial effects of cytokine activation in patients with HF. We can conclude that not all interventions that attenuated LV remodelling had a beneficial effect on survival in clinical trials.

- **Modulators of Nitric oxide- cGMP and signalling pathways**

Nitric oxide (NO) at low concentrations protects cardiomyocytes from ischaemia/reperfusion-injury via soluble guanylyl cyclase activation and cGMP formation and exerts beneficial effects on LV remodelling after myocardial infarction (110).

Takimoto et al. (111) showed that inhibition with cGMP phosphodiesterase-5A, a sustained activation of PKG-I can occur, thus preventing and reversing cardiac hypertrophy and remodeling. Approaches to stimulate NO or its signalling pathways include β-blockers with NO enhancing properties (like nevibolol), or NO-enhancing drugs (112). Whereas low doses of NO produced by eNOS exerts cardioprotective effects, large amounts of NO produced by inducible NO synthase (iNOS) exert detrimental effects and are not necessarily cardioprotective (113, 114).

- **Statins and anti-oxidant strategies**

Several experimental studies showed that statins inhibit cardiomyocyte hypertrophy and enhance endothelial nitric oxide synthase (eNOS) activity. Thus they may play an important role in limiting LV remodeling (110). In an echocardiographic study by Sola et al. (115) in 108 patients with DCM and a LVEF <35%, the use of atorvastatin improved LVEF by approximately

4% and reduced LVDD as compared with placebo. Krum et al. conducted a study with high-dose rosuvastatin in 86 patients with ischaemic or non-ischaemic DCM and did not observe a significant change in LVEF or LVDD (116). In the CORONA Trial rosuvastatin did not significantly reduce the primary endpoint of death from cardiovascular causes, nonfatal myocardial infarction, or non-fatal stroke, nor reduce the number of deaths from any cause. Nevertheless, it reduced cardiovascular hospitalizations (117). A low dose of rosuvastatin was used which possibly was not sufficient to produce the myocardial effects required for an impact on LV remodeling.

In the OPT-CHF study, Hare et al. have examined the effects of oxipurinol in 402 HF-reduced EF patients receiving optimal medical therapy, but the benefits only occurred in patients with elevated serum uric acid and in relation to the degree of uric acid reduction (118).

- **Modulators of inflammation and pro-inflammatory cytokines**

A major challenge to prevent or reverse LV remodeling is to limit detrimental inflammatory cell-mediated changes, while simultaneously maintaining adequate and appropriate LV repair responses (110). The initial remodelling phase after myocardial infarction leading to a removal of necrotic debris and to scar formation (infarct healing) should probably be considered beneficial as it serves to maintain LV structural integrity and to prevent LV rupture. Interference with the process of scar formation during the acute post-myocardial infarction period, by administration of glucocorticosteroids and non-steroidal anti-inflammatory drugs can cause increased thinning of the infarct zone and potentially greater degrees of infarct expansion. Toll-like receptors (TLRs), primary innate immune receptors that are also activated by endogenous signals, are expressed by cardiomyocytes and vascular cells and two experimental studies have observed that TLR-4 activation, that is increased in HF, is an important mediator of maladaptive LV remodelling and dysfunction (119, 120). Another experimental study has shown that exogenous administration of a recombinant human interleukin (IL)-1 receptor antagonist (anakinra) can reduce cardiomyocyte apoptosis and LV remodelling after acute myocardial infarction (121).

- **Metalloproteinase inhibitors**

The PREMIER (Prevention of Myocardial Infarction Early Remodelling) trial included 253 patients with ST-segment elevation MI (LVEF<40%). Patients were randomized to placebo or the oral MMP inhibitor PG-116800, a drug that previously showed significant anti-remodeling effects; after 90 days of follow-up no significant effects on LV remodelling or clinical outcome were noted (122). An experimental study by Spinale et al. has demonstrated that MMP inhibition conferred a beneficial effect on survival early post-myocardial infarction, but that prolonged MMP inhibition was associated with higher mortality rates and adverse LV remodeling, suggesting that there may exist an optimal time window with respect to pharmacological interruption of MMP activity after an acute infarct (123). In support of this concept, Kelly et al. have observed a biphasic profile of plasma MMP-9 that is related to LV remodeling: higher early levels of MMP-9 were associated with the extent of LV remodeling,

but higher plateau levels late after myocardial infarction were associated with a relative preservation of LV function (124).

- **Pro-angiogenetic factors and/or cell transfer**

Bone marrow cells and hematopoietic stem cells have been implanted into the adult's heart, either by intracoronary injection, direct myocardial injections, or mobilized from the periphery through the administration of granulocyte colony-stimulating factors; but recent studies have question the ability of stem cells to differentiate in cardiac myocytes (125-127). Recent evidence has demonstrated the existence of progenitor cells resident within the myocardium and cardiomyocytes capable of re-entering the cell cycle, findings that contradict the traditional idea that the heart is a strictly postmitotic organ (2).

The Myoblast Autologous Grafting in Ischemic Cardiomyopathy (MAGIC) trial demonstrated no significant benefit from this therapy, coupled with an increased risk of malignant ventricular arrhythmias (128). In Stem Cell Infusion in Patients With Ischemic Cardiomyopathy (SCIPIO) study, LVEF increased from 30.3% to 38.5% at 4 months after infusion; cardiac MRI measurements of infarct size demonstrated 24% and 30% decreases at 4 and 12 months, respectively (129). The Cardiosphere-Derived Autologous Stem Cells to Reverse Ventricular Dysfunction (CADUCEUS) trial showed significant reductions in scar, increases in viable heart mass, and improvements in regional contractility and regional systolic wall thickening evaluated by cardiac MRI at 6 months; however, changes in LVDV, LVSV, and LVEF did not differ between groups (130). In the Pilot Study of the Comparative Safety and Efficacy of Transendocardial Injection of Autologous Mesenchymal Stem Cells Versus Allogeneic Mesenchymal Stem Cells in Patients With Chronic Ischemic Left Ventricular Dysfunction Secondary to Myocardial Infarction (POSEIDON), allogeneic mesenchymal stem cells therapy was associated with an improvement in functional capacity; however, neither approach improved exercise VO2max; allogeneic or/and autologous MSCs reduced mean infarct size, sphericity index and LVDV but did not increase EF (131).

3.2. Resynchronization Therapy

Patients with asynchronous wall motion due to intraventricular conduction delay are at increased risk for exacerbated HF. Biventricular pacing or CRT can re-coordinate contraction, thereby acutely improving systolic ventricular function and energetic efficiency (132). The mechanisms for LVRR with CRT remain to be understood, but may comprise: reduced wall stress due to reduced inhomogeneities in regional contractile activation, decreased sympathetic nerve activity, increased metabolic efficiency (132, 133). In animal studies, CRT therapy can restore intracellular Ca2+ homeostasis, normalize the ratio of β1/β2 adrenergic receptors, and alter the expression of genes involved in mitochondrial energetics, ECM and reduce myocardial stress (134-136).

Reverse remodeling with CRT was observed in the MIRACLE study, a prospective, double-blind randomized, in 323 HF patients (mean EF 24±7%) on optimized medical heart failure therapy; CRT induced significant and progressive reverse remodeling over 6 months:

LVDV and LVSV as well as mitral regurgitation decreased, associated with an increase in LVEF; in addition, LV mass decreased and myocardial performance and LV sphericity index improved (69). MIRACLE reported beneficial effects on NYHA symptom class and 6-minute walk test, reductions in LV volumes were found to be dependent on disease type, with greater reductions noted in nonischemic patients versus ischemic patients (137).

Other trials, like the CARE-HF (Cardiac Resynchronization in Heart Failure) (138) and MADIT-CRT (Multicenter Automatic Defibrillator Implantation Trial–Cardiac Resynchronization Therapy) (71) showed that CRTs can improve symptoms and reduce mortality. These results were associated with improvements in LV remodelling and in LV systolic and diastolic function (139). Contrary to MIRACLE study, reductions in mortality and improvements in cardiac function in CARE-HF were similar in nonischemic and ischemic patients (138). In order to optimize patient selection and identify CRT responders a multicenter trial (Predictors of Response to CRT [PROSPECT]) was conducted, enrolling 498 patients, but no specific parameter conclusively improved patient selection criteria (140).

3.3. LV assist device

The most informative data on reverse remodelling are derived from studies in the setting of LVAD support. LVADs showed to be able to increase LV wall thickness, decrease LV volume and induce a favourable leftward shift in the LV pressure-volume curve (141). A significant increase in contractility was demonstrated in cardiac myocytes isolated from hearts that have undergone LVAD support compared with myocytes isolated before LVAD support. In addition to these changes in cardiac myocytes, changes in the ECM and microvascular density (angiogenesis) were also noted. Support with a LV assist device was shown to improve the force-frequency relationship of isolated strips of ventricular tissue, along with improvements in genes encoding for proteins involved in Ca2+ handling (SERCA 2a, the ryanodine receptor, and the sarcolemma sodium-calcium exchanger)(142). LV assist device support led to a restoration of the integrity of the dystrophin cytoskeleton, which had been shown to be disrupted in myocytes from failing hearts (143).

Treatment with β-blockers and LVAD support decreased hyperphosphorylation of the ryanodine receptor, which has been implicated in a calcium leak from the sarcoplasmic reticulum in failing hearts and hence contractile dysfunction. However, tissue levels of angiotensin II, a profibrotic peptide, increased in patients supported by LVADs, who were not medicated with a ACEI (22).

Gene expression profiling studies have shown that only 5% of genes that are dysregulated in failing hearts revert to normal after LVAD support, despite morphological and functional improvement (144, 145). Although maximal calcium-saturated force generation is improved in myocytes after LVAD support, force generation is still less than in myocytes from nonfailing controls, despite reversal of cardiac myocyte hypertrophy (146). After LVAD, ECM does not revert to normal and increased myocardial fibrosis can occur.

3.4. Surgical approaches

A number of different surgical approaches have been tried to prevent and/or retard LV remodeling, including surgical myoplasty (147), which has been abandoned, mitral valve surgery (148) volume reduction surgery (partial left ventriculectomy) (149) and endoventricular circular patch plasty/surgical anterior ventricular restoration Dor procedure (150).

The CorCap device has been implanted worldwide in more than 130 patients with dilated cardiomyopathy (idiopathic or ischemic). During follow-up, chamber dimensions decreased, and ejection fraction and NYHA class improved (151). The Acorn Trial demonstrated sustained reverse remodeling of the LV and the long-term safety and efficacy of the CorCap cardiac support device as an adjunctive therapy for patients with HF who remain symptomatic despite optimal medical therapy (152, 153).

3.6. Recurrence of LV systolic dysfunction

Withdrawal of β-blockers and ACEI after LVRR may be associated with recurrence of systolic dysfunction (154) or left ventricular hypertrophy (155). In a study, involving 42 patients in whom LVEF recovered, LV systolic dysfunction reappeared during the follow-up period in eight of the 42 patients (19.0%). Patients in whom LV dysfunction recurred had discontinued neuro-hormonal blocker medication (62.5% versus 5.9%, $P<0.05$)(59). Cardiac ultrastructural changes, like interstitial fibrosis, can be risk factor for recurrent HF.

Studies are needed to define what kind of neuro-hormonal blockade should be maintained after recovery of LV function.

4- CLINICAL INVESTIGATION AT HEART FAILURE AND TRANSPLANT CLINIC

Previous clinical studies performed at the HF and Transplant Clinic of the Cardiology Department of Centro Hospitalar São João provided the background of the present investigation.

The most important determinants of the improvement in the prognosis of DCM observed over the past years were: 1) the implementation of familial screening programs for DCM that have enabled earlier diagnosis; 2) the systematic implementation of evidence based medical and device therapies that promote LVRR.

We verified a change in prognosis in the last 15 years. In a group of 219 patients with DCM of non-ischemic aetiology (about 80%), with mean LVEF of 24 ± 9%; NYHA class II or III, the survival of the 135 patients admitted before 1996 was lower than that of patients admitted later, coinciding with the beginning of the prescription of ß-blockers and mineralocorticoid antagonists, with no difference between the use of ACEI (156).

In another study, patients with alcohol-related DCM (157), recovery occurred in 36% of 45 patients. Those who improved LV ejection fraction were more frequently abstinent (75% vs 28%, $p < 0.01$), were in NYHA class I or II at the end of follow-up (100% vs 69%, $p = 0.02$) and had better survival (100% at 5 years and 80% at 10 years vs 64% at 5 years and 32% at 10 years, $p < 0.01$). Patients with non-abstinent alcoholic DCM had a higher mortality than those with abstinent alcoholic DCM and tended to have a worse prognosis than patients with idiopathic DCM. The predictors of death or transplantation in patients with alcoholic DCM were the absence of alcohol withdrawal and the lower use of β-blocker. The predictors of death or transplantation in patients with idiopathic DCM were female gender, higher heart rate, lower body mass index, and lower β-blocker use (158) (Full text at Appendix I, published only in abstract form).

In a retrospective study including 62 patients with idiopathic DCM, a significant improvement of LVEF occurred in 11 (18%) patients. Patients in whom LVRR occurred were younger at the time of diagnosis, had a lower heart rate and had lower diastolic diameters than those in whom it did not (159).

It has been postulated that patients with non-ischemic cardiomyopathy are more likely to recover from LV dysfunction. In those patients, there is a pronounced adrenergic activation for an equivalent degree of myocardial dysfunction, but with a higher viable (non-necrotic) myocardium. A marked reduction in sympathetic activity appears to reduce mortality. The magnitude of heart rate reduction, rather than its baseline level, appears to be associated with a higher increase in LVEF (160). To corroborate this, we evaluated the effects of β-blockers on the recovery of LVEF in 30 patients (161) with DCM, a group (A) evaluated before and after carvedilol up to the maximum tolerated dose, and a group Control (B) patients with HF and β-blocker intolerance. In group A, before and after the use of carvedilol, mean values of heart rate, ejection fraction, baroreceptor gain, HR variability in the high frequency domain were significantly lower (162) (163).

Another study of adrenergic activity with 123 I-MIBG in our HF clinic, in patients with familial DCM (164-166), showed that there is a lower value of cardiac/ medullary uptake rate and a higher rate of myocardial washout in patients With MCD phenotype relative to those without MCD phenotype. In some families with isolated LV abnormalities or with normal echocardiography (including 3 patients with reverse remodeling), high rates of myocardial washout were also found, suggesting that cardiac MIBG may reveal early changes in adrenergic activity.

Regarding functional class and physical performance, the maximum VO2 value of the cardio-respiratory effort test (CPET) plays a fundamental role in the prognosis of HF patients. In a study (167), new variables of the cardio-respiratory effort test in dilated cardiomyopathy were studied, because the peak VO2 can be underestimated if the test is submaximal, common in the patient with HF. In 83 consecutive days with MCD, the predictive parameters of death or transplantation were VO2 peak, VO2 lean IMC> 30, dVE/ VCO2, dVE /VCO2 /VO2p, O2 pulse (%), circulatory power, percentage of VO2 at anaerobic threshold (AT), peak O2 consumption (POUE) efficiency and POUE in AT. The POUE and POUE in the LA were the parameters that showed greater protective effect, they represent the relationship between minute ventilation and oxygen uptake during exercise and incorporate cardiovascular and pulmonary factors, being relatively insensitive to the duration of exercise.

Finally, to establish the prognosis of HF, peak VO2 and other prognostic scores such as the Heart Failure Survival Score (HFSS) have extensively studied. However, this score was validated in an era in which patients were not treated with β-blockers or with CRT/ICD. In our population (168) we sought to validate the current prognostic value of HFSS in 70 patients and to study other prognostic variables. The HFSS contributed to risk stratification, the use of β- blockers did not modify survival in the high risk and low risk groups of HFSS, but improved survival in the medium risk group: 100% at 1 year, 78% at 5 years, 65% at 10 years. Device use (27% of cases) was not significantly associated with improved survival. In addition to the parameters obtained for the calculation of HFSS, the predictors of death or urgent transplantation were the presence of elevated jugular venous pressure, S3 presence, right ventricular dysfunction, larger left atrial size, greater LVDD, greater LV mass index, higher PSAP and higher BNP. It is concluded that the addition of clinical/ hemodynamic parameters, parameters of ventricular and atrial remodeling, right ventricular function and BNP value may improve the efficacy of established prognostic scores.

CHAPTER III

AIMS

AIMS

- Although several reviews have summarized the changes that occur at the cellular, molecular, and anatomic level during cardiac remodeling, fewer studies have focused on the factors that allow the heart to revert to normal LV size and shape (i.e., reverse remodeling).

- Clarifying the mechanisms of reverse remodeling leading to sustained functional myocardial recovery will have a dual benefit: (a) it will enable changes in the therapeutic strategy by modifying the duration and intensity of treatment, aiming to promote myocardial recovery and (b) it will allow to identify reliable predictors of recovery.

- The great majority of clinical reports on myocardial recovery found in the literature refer to cases occurring after transient injury (e.g., viral infection, inflammation, toxic injury) rather than after long-standing and/or permanent injury (e.g., myocardial infarction, genetic abnormalities). This is consistent with the view that the ability of the heart to "recover" is related to the injury nature and to the extent of the subsequent myocardial damage. Therefore, in this prospective study we aimed to evaluate recovery of left ventricular function and the reversal of ventricular remodeling in patients with more long-standing Heart Failure, i.e., patients with chronic Idiopathic Dilated Cardiomyopathy (DCM), after optimized medical therapy.

- The predictors of left ventricular reverse remodeling (LVRR) in response to a specific therapeutic strategy need to be defined. In our population we evaluated the prevalence of LVRR, and the clinical, echocardiographic, biomarker, cardiopulmonary stress test (CEPT) predictors of the occurrence of LVRR. We included cardiac MRI in the evaluation protocol. This exam may indirectly assess contractile reserve, which may be inversely proportional to the extent of myocardial fibrosis.

- In previous clinical trials most, but not all, interventions producing LV reverse remodeling translated into a beneficial effect on survival. A secondary aim of our research was to evaluate the impact of reverse remodeling on prognosis in the setting of Idiopathic DCM.

- Cardiomyocyte shortening improvement alone cannot provide a normal LVEF. Only appropriate LV shape (ellipsoid) and orientation of the myocardial fibres in a helicoidal or spiral form can achieve this goal. Non-contractile myocardial components contribute to the appropriate spatial orientation and the mechanical stability of cardiomyocytes and are essential for the maintenance of a normal LVEF.

- Myocardial deformational parameters remain incompletely characterized in patients with DCM. LVEF does not correlate with patient symptoms, a normal LVEF does not exclude nor imply diastolic dysfunction, and LVEF cannot be used as a substitute for cardiac output. The changes in LV myocardial fibre length and thickness (myocardial

deformation or strain), which are fundamental component of LV dynamics, can be assessed with speckle tracking echocardiography. Therefore, we aim to evaluate detailed echocardiographic parameters in patients with DCM, during LVRR.

- Cardiac extracellular matrix (ECM) is a dynamic structure and its turnover depends on a fine balance between matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs). The ECM plays an important role in the distribution of mechanical forces throughout the heart and, thus, it is crucial for maintenance of normal ventricular pump function (169). The deformation of the myocardial matrix during systole results in storage of mechanical energy, turning into a rapid untwist and recoil, generating diastolic suction that is important for early rapid filing of the LV.

- The biochemical, structural, and functional roles of ECM changes remain the most poorly understood aspect of heart remodeling and reverse remodeling. As a final purpose, we evaluated emerging biomarkers in LVRR, mostly markers of ECM remodeling. We also measured others circulatory molecules related to biological pathways involved in HF, such as markers of neuro-hormonal blockade, inflammation and apoptosis.

CHAPTER IV

METHODS

METHODS

1. Inclusion criteria

a) All patients included into this study had a diagnosis of idiopathic DCM defined by a left ventricular ejection fraction (LVEF) < 40%, left ventricular diastolic diameter (LVDD) > 33 mm/m2 in men or > 32 mm/m2 in women).

b) All were followed at the HF and Transplant Clinic of Cardiology Department, were above 18 and were included consecutively.

c) All patients had the diagnosis established between 2 to 3 years before inclusion and had with two initial values of LVEF of <0.40 more than one year apart one from each other.

d) In all cases the plausible etiology was unknown.

2. Exclusion criteria

a) We excluded patients with left ventricular dilatation and dysfunction due to coronary artery disease defined by the presence of one of the following: history of myocardial infarction or angina pectoris, the presence of more than 50% diameter narrowing in any of the major coronary arteries or their branches, or myocardial perfusion abnormalities detected by SPECT or cardiac MRI.

b) We also excluded patients with other causes of DCM, such as: history of moderate or severe hypertension; diabetes mellitus with end-organ damage or on insulin therapy, primary mitral or aortic valvular disease of at least moderate degree; heavy alcohol consumption (>100 g/day), chemotherapy-induced left ventricular dysfunction and peripartum cardiomyopathy.

c) Patients with a history of uncontrolled atrial and ventricular arrhythmias were excluded.

d) We excluded patients with acute HF with a positive biopsy for acute myocarditis, or with a positive serology for acute phase of bacterial or viral infection, or with a cardiac MRI compatible with acute myocarditis.

d) We excluded patients with atrial fibrillation in a latter cohort, since it could introduce errors in the assessment of LVEF.

3. Definition of Left ventricular reverse remodeling

In this study, left ventricular reverse remodeling (LVRR) was defined by the simultaneous presence of the following conditions:

a) the occurrence during follow-up of an absolute LVEF increase ≥ 10 percentage units (for instance a LVEF increase from 30% to 40%) comparing to baseline. This had to be observed in at least two consecutive echocardiograms separated by more than 6 months,

b) a concomitant decrease in LVDD,

c) the increase in LVEF occurred in the absence of CRT or mechanical ventricular assistance.

4. Assessment of remodeling

Two dimensional-echocardiography using the Simpson's rule is considered the current standard for LVEF and was the imaging method we used to evaluate the changes in LVEF in our patients. Similarly, changes in LV volumes were assessed by 2D-echocardiography.

Geometric abnormalities leading to loss of cardiomyocyte orientation underlie the decrease of LVEF in eccentric hypertrophy (171). So, we measured LV sphericity index which is a major determinant of LV twist and consequently of LVEF.

We also assessed other important echocardiographic parameters:

- Degree of mitral and tricuspid regurgitation by Doppler,
- Pulmonary artery systolic pressure (PSAP),
- Left ventricular mass,
- Early diastolic (E) and atrial (A) wave velocities, E/A ratio,
- E-wave deceleration time, spectral pulsed-wave Doppler–derived early diastolic velocity (e´) and E/e´ratio,
- Left ventricular myocardial performance index (LV Tei index),
- Right myocardial performance index (RV Tei index),
- Circumferential strain rates - assessed from basal, mid, and apical LV short-axis views,
- Longitudinal strain rate - assessed from the basal, mid, and apical levels in apical four-chamber, two-chamber, and long-axis views.

5. Baseline evaluation

Baseline evaluation consisted of:

- History and physical examination.
- Electrocardiogram, 24-hour EKG.
- General blood chemistry.
- Transthoracic 2D-echocardiogram.
- Cardiopulmonary stress test (CPET) with the determination of peak VO2, dVE/VCO2, dVE/VCO2/VO2p, O2 pulse (%), circulatory power, percentage of VO2 at anaerobic threshold (AT), peak O2 consumption (POUE) efficiency and POUE in AT,
- Determination of Heart Failure Survival Score (HFSS).
- Measurement of serum biomarkers:
    - Available biomarkers, reflective of diverse biological pathways in HF:
        - Adrenalin, noradrenalin (neurohormonal activation)
        - Plasma renin, aldosterone (neurohormonal activation)
        - B-type natriuretic peptide (BNP) (neurohormonal activation)

- High-sensitivity C-reactive protein (hsCRP) (inflammation)
- Cancer antigen CA-125 (inflammation)
- Uric acid and Lp(a) (oxidative stress)
- Creatinine and cystatin C (renal function)
- 25-OH-vitamin D (extracellular remodeling).

- Novel biomarkers:
  - Markers of ECM remodeling:
    - Matrix metalloproteinase-3 (MMP-3)
    - Tissue inhibitor of matrix metalloproteinase- 2 (TIMP-2)
    - Protein ST2 (receptor for interleukin 33)
    - Galectin-3
  - Markers of inflammation:
    - Soluble TNF receptor I/TNFRSF1A (sTNF-R1iii)
  - Marker of apoptosis:
    - Growth-differentiation factor 15 (GDF-15).

- Cardiac MRI, with quantification of the extent of LGE, quantified by the number of segments affected.

6. Follow-up

During follow-up, the patients were managed according to the European Society of Cardiology Heart Failure Guidelines and the maximal tolerated doses of the disease modifying drugs were administered.

2D-echocardiograms were performed every 3 to 6-months and the occurrence of LVRR was assessed according to the above definition.

Patients who received a CRT were considered to have no LVRR, so we analyzed the last echocardiographic data before the implantation of the device.

CHAPTER V

RESULTS

1. Prevalence, predictors and prognosis of reverse ventricular remodeling in idiopathic dilated cardiomyopathy

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ORIGINAL ARTICLE

Prevalence, predictors and prognosis of ventricular reverse remodeling in idiopathic dilated cardiomyopathy

Sandra Amorim*, Manuel Campelo*, Elisabete Martins, Brenda Moura, Alexandra Sousa, Teresa Pinho, José Silva-Cardoso*, Maria Júlia Maciel

Cardiology Department, Hospital de São João, Porto, Portugal

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Abstract
Introduction: Cardiac remodeling is manifested as changes in size, shape and function of the heart. We studied the prevalence, prognosis and predictors of left ventricular reverse remodeling (LVRR) in idiopathic dilated cardiomyopathy (IDCM) after optimized medical therapy.
Methods: A total of 113 IDCM patients were followed for 7.1±5.6 years. LVRR was defined as an increase of 10 units in ejection fraction (EF) and decrease in left ventricular diastolic diameter (LVDD), in the absence of resynchronization therapy.
Results: Baseline EF was 27±8% and LVDD index was 37.1±6.3 mm/m^2. LVRR occurred in 34.5% within 22.6 months. Final EF was 47.5±10.1%, LVDD index was 30.2±3.9 mm/m^2. LVRR was associated with better NYHA class (I–II) and lower BNP ($p<0.01$) and all patients were alive.

Univariate predictive factors of LVRR ($p<0.05$) were mild hypertension, atrial fibrillation, ventricular hypertrophy on ECG, absence of left bundle branch block, shorter QRS duration, higher hematocrit, lower LVDD index, higher peak oxygen uptake efficiency (VO_2/log 10[VE]) and lower $dVE/VCO_2/VO_2$, treatment with angiotensin-converting enzyme inhibitors (ACEI)/angiotensin receptor blockers (ARB) and use of maximal doses of ACEI/ARB and beta-blockers. Multivariate regression analysis showed that higher doses of ACEI/ARB (OR: 0.32, 95% CI 0.11–0.92) were independently associated with LVRR. Non-transmural late enhancement on cardiac MRI was not a predictor of LVRR.
Conclusions: LVRR occurred in one third of IDCM patients, especially in those with mild hypertension and with less advanced disease, who may have benefited from maximal drug titration.

* Corresponding authors.
E-mail address: sandra.maria.amorim@netcabo.pt (S. Amorim).

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PALAVRAS-CHAVE
Miocardiopatia dilatada;
Remodelagem reversa;
Prognóstico

Prevalência, preditores e prognóstico da remodelagem reversa na miocardiopatia dilatada idiopática

Resumo
Introdução: A remodelagem ventricular é caracterizada por alterações no tamanho, forma e função do coração. Estudámos a prevalência, o prognóstico e os fatores preditores de reversão da remodelagem do ventrículo esquerdo (RRVE) na miocardiopatia dilatada idiopática (MCDI), após a terapêutica farmacológica otimizada.
Métodos: Cento e treze doentes foram seguidos durante 7,1±5,6 anos. A RRVE foi definida como um aumento de dezunidades da fração de ejeção (FE) e diminuição do diâmetro diastólico do VE (VED), na ausência de terapêutica de ressincronização.
Resultados: A FE basal foi de 27±8% e o VED de 37,1 ± 6,3 mm/m^2. A RRVE ocorreu em 34,5% dentro de 22,6 meses. A FE final foi de 47,5 ± 10,1%, o VED *index* foi de 30,2±3,9 mm/m^2. A RRVE associou-se a melhor classe NYHA (I-II), menor BNP e a mortalidade nula.
Os preditores de RRVE foram hipertensão arterial (ligeira), fibrilhação auricular, hipertrofia ventricular esquerda (no ECG), ausência de bloqueio de ramo esquerdo, menor duração do QRS, maior hematócrito, menor VED *index*, melhor eficiência de oxigénio no pico do exercício (VO2/LG10[VE]), um menor DVE/VCO2/VO2, uso de IECA/ARA-II e uso de doses máximas de IECA/ARA-II e bloqueadores-β. Na análise multivariada o uso de doses máximas de IECA/ARA-II (OR: 0,32, 95% CI 0,11-0,92) foi um preditor independente. A presença ou extensão do realce tardio na RMN cardíaca não foi preditora de RRVE.
Conclusão: A RRVE ocorreu num terço dos pacientes MCDI, naqueles com hipertensão ligeira e com doença menos avançada, que poderão ter beneficiado da máxima titulação dos fármacos.

Introduction

Cardiac remodeling is defined as genome expression resulting in molecular, cellular and interstitial changes and manifested clinically as changes in size, shape and function of the heart.[1] The progression of heart failure (HF) is associated with left ventricular (LV) remodeling, which manifests as gradual increases in LV end-diastolic and end-systolic volumes, wall thinning, and a change in chamber geometry to a more spherical, less elongated shape, with a progressive decrease in ejection fraction (EF).

When ventricular remodeling is advanced, it begins to be self-sustaining and capable of driving disease progression, regardless of the patient's neurohormonal status. This may explain why medical therapies lose their effectiveness in end-stage HF, and why some device-based therapies (cardiac resynchronization and mechanical ventricular assistance), which can affect LV remodeling, are beneficial.

The overall importance of ventricular remodeling as a pathogenic mechanism and prognostic determinant is not clear. Some drug therapies and cardiac devices that increase the survival of patients with HF can slow, and in some cases even reverse, certain parameters of remodeling. Controversially, as in the case of etanercept[2] and in cardiac resynchronization,[3,4] reverse remodeling has not translated into increased survival. Additionally, the molecular mechanisms of reverse remodeling have not been fully elucidated.

Left ventricular reverse remodeling (LVRR) is characterized by a decrease in LV dimensions, normalization of LV shape and improvement of systolic function.

A significant prevalence of recovery of LV function in patients with dilated cardiomyopathy (DCM) has been reported.[5] However, such studies included patients with new-onset DCM like acute myocarditis, and other reversible causes of DCM, such as peripartum and alcohol-related DCM. The mechanisms underlying LVRR in such situations appear to be different from those involved in chronic idiopathic DCM.

The aim of this prospective study was to assess recovery of LV function and reversal of ventricular remodeling in patients with chronic idiopathic DCM, after optimized medical therapy. We set out to assess its prevalence, to identify its predictors and to determine whether it was associated with better prognosis.

Methods

The study included consecutive adult patients with idiopathic DCM (left ventricular diastolic diameter [LVDD] >33 mm/m^2 in men, >32 mm/m^2 in women) between 2000 and June 2012 followed in an HF clinic, diagnosed less than 24 months previously and with two initial values of left ventricular ejection fraction (LVEF) of <0.40 more than one year apart.

We excluded DCM patients with secondary etiologies, including a history of myocardial infarction or angina, those with ischemia or significant coronary disease on coronary angiography, a history of moderate or severe hypertension, at least moderate primary mitral or aortic valvular disease, heavy alcohol use (>100 g/day), chemotherapy-induced and peripartum cardiomyopathy, acute HF with biopsy positive

for acute myocarditis or positive serology for acute bacterial or viral infection. We included patients with idiopathic DCM, diagnosed after respiratory infections but with LV dysfunction that persisted for over a year (in order to exclude myocarditis). We also excluded patients with uncontrolled atrial and ventricular arrhythmias.

At baseline, patients underwent clinical assessment, electrocardiogram (ECG), 24-hour ECG, transthoracic echocardiogram, blood laboratory measurements, cardiopulmonary exercise testing (CPET) and cardiac magnetic resonance (CMR).

Patients were managed according to current clinical practice guidelines and clinicians aimed to reach the recommended target doses for all therapies.

During follow-up, periodic clinical assessment, laboratory measurements and echocardiogram were performed.

This study was in accordance with the recommendations set by the Declaration of Helsinki and with local legal requirements.

Definition of left ventricular reverse remodeling

LVRR was defined as an absolute increase on two consecutive echocardiograms more than six months apart of 10 units of LVEF, together with a decrease in left ventricular diastolic diameter (LVDD), without worsening of mitral regurgitation, in the absence of cardiac resynchronization therapy (CRT) or mechanical ventricular assistance.

Transthoracic echocardiography protocol

Transthoracic echocardiography was performed at baseline and during follow-up using two commercially available systems: General Electric Vivid 3.0 and Vivid 7.0 with a 2.5-MHz transducer. The following parameters were measured according to the standards defined by the American Society of Echocardiography and the European Association of Echocardiography[6]: LVDD and end-systolic diameter; LV EF (%) calculated by Simpson's biplane method; degree of mitral regurgitation by Doppler and color Doppler, on a scale from 0 to 4; left atrial diameter; LV posterior wall thickness and interventricular septal thickness; right ventricular systolic dysfunction (defined as tricuspid annular systolic excursion [TAPSE] <16 mm); and pulmonary artery systolic pressure (PASP) calculated by tricuspid velocities. Data on diastolic function were incomplete.

Patients who received CRT were considered have no LVRR, so EF and LVDD before CRT were included in the analysis.

All data were digitally stored, and off-line data analysis was performed by two echocardiography specialists, blinded to the study.

Cardiopulmonary stress testing

Patients underwent maximal symptom-limited CPET (Jaeger Oxycon Mobile 4.6). Blood pressure was measured manually and a modified Bruce protocol was used. All tests were interrupted due to symptoms. Expired ventilatory flow (VE), oxygen uptake (VO_2), carbon dioxide output (VCO_2) and other cardiopulmonary variables were acquired breath-by-breath by pneumotachograph with bidirectional differential pressure. Peak oxygen uptake (VO_2 peak) was calculated as the mean values during the last 30 s of effort. The anaerobic threshold (AT) was calculated automatically by the V-slope method. We also determined circulatory power (VO_2 peak$\times$peak systolic blood pressure), VE/VCO_2 slope, ventilatory equivalent for oxygen (VE/VO_2) and VE/CO_2 slope normalized for peak VO_2. Because of the limitations of the system, instead of calculating the oxygen uptake efficiency slope, we calculated peak oxygen uptake efficiency (POUE) (peak VO_2/log 10 peak VE) at AT, which is more easily obtained and has similar prognostic value.[7]

The Heart Failure Survival Score (HFSS) was calculated by the equation: (0.0216$\times$heart rate)+(−0.0255$\times$mean blood pressure)+(−0.0464$\times$EF)+(−0.0470$\times$Na+ concentration)+(−0.0546$\times$peak VO_2)+(0.6083$\times$QRS>120 ms 1, no 0)+(0.6931$\times$ischemic etiology 1, no 0).

Cardiac magnetic resonance

The CMR studies were performed on a 3 T clinical scanner (Siemens® Magnetom Trio). Electrocardiogram-gated cine steady-state free precession imaging was performed in short-axis and orthogonal LV long-axis views. A breath-hold, T2-weighted dark blood sequence was acquired. Late gadolinium enhancement (LGE) images were acquired 10–15 min after gadolinium administration using a phase-sensitive inversion-recovery sequence.

The extent of LGE was quantified by the number of segments affected. The presence and distribution of LGE were independently determined by one radiologist and one cardiologist, blinded to the study.

Statistical analysis

All values are reported as mean ± SD, median ± interquartile range or percentages according to data characteristics. Differences between subjects in each arm were assessed using the chi-square test for categorical variables and the Student's t test or the Mann-Whitney test for continuous variables, as appropriate. A two-tailed $p<0.05$ was considered to indicate statistical significance.

To assess predictors of LVRR from baseline characteristics and from therapy, univariate analysis included all relevant clinical or laboratory parameters. Variables with $p<0.05$ from the univariate analysis were entered in multivariate Cox regression analysis, but variables with low quantities of data (those from 24-hour ECG, CPET and CMR) were excluded.

Results

Population characteristics

A total of 113 patients were included, followed for 7.1±5.6 years, mean age 50±14 years; 74 were male (66%).

At baseline, mean EF was 27±8%, LVDD was 67±9 mm, LVDD index was 37.1±6.3 mm/m^2 and grade >II mitral regurgitation was present in 34% of patients.

Table 1 Baseline characteristics of the study population at baseline (n=113).

Age (years)	50.1±14.5
Male (%)	65.5
Body mass index	27.1±3.9
Hypertension (%)	39.8
Diabetes (%)	17.7
Chronic pulmonary disease (%)	8.8
Moderate alcohol intake	21.2
Heart rate (bpm)	80.2±17.7
Systolic blood pressure (mmHg)	119±20
NYHA class I (%)	20.4
NYHA class II (%)	69.0
NYHA class III (%)	8.8
NYHA class IV (%)	1.8
Atrial fibrillation (%)	14.2
LBBB (%)	44.2
QRS duration (ms)	126.6±31.9
LV hypertrophy (%)	21.2
Echocardiography	
RV dilation (%)	11.5
RV dysfunction (%)	8.0
TAPSE (mm)	23.4±4.5
Grade >II/IV tricuspid regurgitation	5.4
PASP (mmHg)	39.6±16.0
LVDD (mm)	67.0±8.7
LVSD (mm)	57.0±8.1
LVDD/BSA (mm/mm^2)	37.1±6.4
Ejection fraction (%)	27.2±8.2
LV mass/BSA (g/m^2)	185.1±30.4
Grade >II/IV mitral regurgitation (%)	33.6
Left atrial diameter (mm)	45.7±6.6

BSA: body surface area; LBBB: left bundle branch block; LV: left ventricular; LVDD: left ventricular end-diastolic diameter; LVSD: left ventricular end-systolic diameter; NYHA: New York Heart Association; PASP: pulmonary artery systolic pressure; RV: right ventricular; TAPSE: tricuspid annular systolic excursion.

On ECG, 44% had left bundle branch block (LBBB), 46% had LV conduction disturbances and 14% had atrial fibrillation. The majority of patients were in NYHA class II (69%). Table 1 details the patients' baseline clinical characteristics.

At the end of follow-up, 90% were treated with angiotensin-converting enzyme inhibitors (ACEI)/angiotensin receptor blockers (ARB), 64% with beta-blockers, 30% with aldosterone antagonists and 33% with digoxin. Optimal recommended doses of ACEI/ARB were reached in 52.2% (20–30 mg lisinopril, 5–10 mg perindopril, 16–32 mg candesartan) and optimal doses of beta-blockers were reached in 47.8% (25–50 mg bid carvedilol, 5–10 mg bisoprolol). Figure 1 shows therapy at baseline and at the end of follow-up.

Urgent heart transplantation or death occurred in 16% of patients (nine deaths, nine transplantations), 38% were hospitalized for worsening HF and 30% had cardiac devices implanted: implantable cardioverter-defibrillator (ICD) in 19%, CRT plus ICD in 8%, and CRT pacing in 3%.

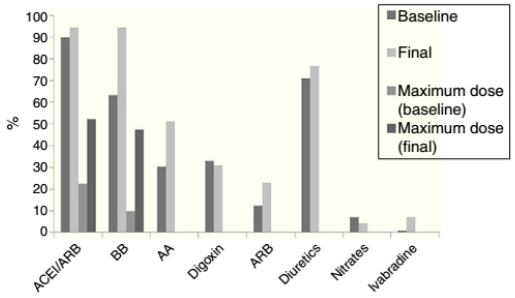

Figure 1 Medical therapy at baseline and at the end of follow-up. AA: aldosterone antagonists; ACEI: angiotensin-converting enzyme inhibitors; ARB: angiotensin receptor blockers; BB: beta-blockers.

Prevalence and prognostic value of left ventricular reverse remodeling

Initial EF in patients who recovered LV function was 28±9%, not significantly different from the 27±9% in those who did not recover.

LVRR occurred in 39 patients (34.5%) within 22.6 months (median). Final EF was 47.5±10.1% (Δ EF 19.4±9.0%), LVDD was 55.7±6.7 mm (Δ LVDD −9.6±−7.4 mm), LVDD index was 30.2±3.9 mm/m^2 and only 3.5% had grade >II MR (Figure 2).

Patients with LVRR had better NYHA functional capacity: class I (67% vs. 25%, p<0.01), class II (43% vs. 31%, p<0.01) and had lower BNP (median 27.4 vs. 160.0 pg/ml, p<0.01), compared with those without LVRR. LVRR was associated with lower rates of HF hospitalization (23.1% vs. 44.6%, p=0.02), cardiac death and urgent transplantation (0.0% vs. 24.3%, p<0.01).

Factors predicting left ventricular reverse remodeling

Because of technical reasons and pre-existing contraindications, only 89 patients underwent 24-hour ECG, only 55 patients underwent CPET and only 38 underwent CMR at baseline.

Variables at baseline that predicted LVRR were (Table 2): mild hypertension (54% vs. 32%, p<0.05), atrial fibrillation (26% vs. 8%, p<0.05), ventricular hypertrophy on ECG (36% vs. 14%, p<0.05), absence of LBBB (31% vs. 51%, p<0.04), shorter QRS interval (117 ms vs. 131 ms, p<0.05), higher hematocrit (43.2 vs. 40.8%, p<0.05), lower LVDD index (35.4 vs. 38.0 mm/m^2, p<0.05) and less non-sustained ventricular tachycardia on 24-hour ECG (12.5% vs. 33.9%, p=0.03).

Predictor variables from CPET were higher POUE (0.879 vs. 0.734, p<0.05) and lower dVE/VCO_2/VO_2 (2.5 vs. 4.0, p<0.05) (Table 3).

Mean calculated HFSS was 8.97±0.85, with 98.2% of patients at low risk and only 1.8% at medium risk, and did not differ in patients who did not recover EF.

Non-transmural LGE (showing midwall fibrosis) on CMR was present in 55.3% of patients; in 26.3% it was limited

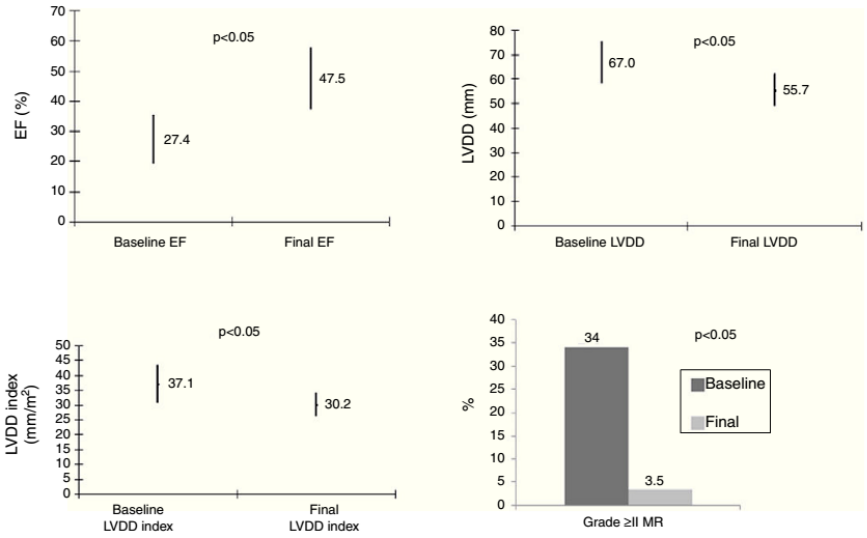

Figure 2 Echocardiographic measures of reverse remodeling. EF: ejection fraction; LVDD: left ventricular diastolic diameter; MR: mitral regurgitation.

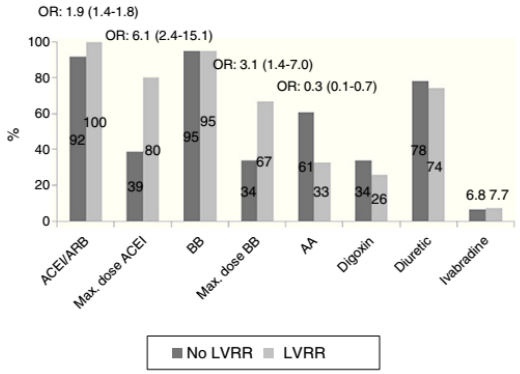

Figure 3 Pharmacological predictors of reverse remodeling during follow-up, showing differences in percentages of medical therapy between patients with and without left ventricular reverse remodeling. AA: aldosterone antagonists; ACEI: angiotensin-converting enzyme inhibitors; ARB: angiotensin receptor blockers; BB: beta-blockers; LVRR: left ventricular reverse remodeling; Max.: Maximum; OR: odds ratio.

to one LV segment and in 28.9% it was observed in more than one segment. LGE or other CMR parameters, such as right ventricular EF, were not predictors of LVRR (Table 3).

During follow-up, patients in the LVRR group were more often treated with ACEI/ARB (100% vs. 92%, p<0.05) and with maximal doses (80% vs. 39%, p<0.01). There were no differences in the use of beta-blockers, but those who had LVRR more often reached maximal doses (67% vs. 34%, p<0.01) and were less often medicated with aldosterone antagonists (33% vs. 61%, p<0.01) (Figure 3).

Multivariate regression analysis showed that only treatment with recommended doses of ACEI/ARB (OR: 0.32, 95% CI 0.11–0.92) was independently associated with LVRR.

Discussion

In the present study, we describe the frequency of improvement in LV systolic function in patients with chronic idiopathic DCM in an unselected population.

LVRR has been described in secondary forms of DCM, such as peripartum cardiomyopathy, alcohol abuse, myocarditis and ischemic heart disease, but the mechanisms underlying such conditions are different from those in idiopathic DCM.[8,9]

A significant prevalence of recovery of LV function has also been described in recent-onset DCM. Those patients have a higher potential for LVRR, due to resolution of the underlying disease, as in myocarditis, or to favorable effects of therapy. Kubanek et al.[10] reported a prevalence of 45% of LVRR at 12 months in 44 patients with recent-onset DCM, including some with active and resolving myocarditis. We only included patients with idiopathic DCM diagnosed less than 24 months previously, but with two initial values of EF of <0.40 more than one year apart, in order to exclude resolving myocarditis.

In our population, LVRR occurred in approximately one third of patients within 22 months of diagnosis. It was associated with improvement in NYHA functional class, with decrease in BNP compared with those who did not recover, and with excellent prognosis.

Recovery in EF and reverse remodeling was associated with maximal treatment with ACEI/ARB and beta-blockers. Patients with LVRR were less often medicated with aldosterone antagonists, probably because they achieved better NYHA functional class.

Table 2 Baseline variables predicting left ventricular reverse remodeling.

	No LVRR (n=74)	LVRR (n=39)	p	OR	CI
Age (years)	49.8±14.5	49.2±13.9	0.84		
Male (%)	64.9	66.7	0.85		
Hypertension (%)	32.4	53.8	0.03	2.4	1.1–5.4
NYHA class I (%)	21.6	17.6	0.91		
Heart rate (bpm)	78.6±16.9	83.3±19.1	0.18		
Systolic blood pressure (mmHg)	117.0±20.7	122.7±18.9	0.16		
Atrial fibrillation (%)	8.1	25.6	0.01	3.9	1.3–11.8
QRS duration (ms)	131.8±32.2	117.1±29.4	0.02	0.9	0.9–0.98
LBBB (%)	51.4	30.8	0.03	0.4	0.2–0.9
LV hypertrophy (%)	13.5	35.9	0.01	3.5	1.4–9.0
Laboratory variables					
Hematocrit (%)	40.8±4.0	43.2±3.1	0.01	1.2	1.1–1.3
Creatinine clearance (ml/min)	99.7±32.9	107.1±29.1	0.24		
Uric acid (mg/dl)	35.7±30.9	40.4±31.1	0.55		
Na+ (mEq/l)	138.6±2.8	139.7±2.6	0.06		
BNP (pg/ml) (median)	65.0±204.8	26.2±1839.0	0.48		
Echocardiogram					
LV ejection fraction (%)	27.0±9.0	28.1±8.7	0.46		
RV dysfunction (%)	8.1	7.9	0.97		
LA diameter (mm)	46.1±7.2	44.8±5.2	0.30		
LA volume/BSA (ml/m^2)	38.1±16.0	37.6±11.3	0.93		
LV diameter (mm)	68.0±9.5	65.1±6.8	0.09		
LV diameter/BSA (mm/m^2)	38.0±7.0	35.4±4.5	0.04	0.9	0.86–0.99
LV mass/BSA (g/m^2)	337.9±109.2	315.8±71.1	0.26		
Grade >II/IV mitral regurgitation (%)	36.5	28.2	0.37		
PASP (mmHg)	39.9±17.1	39.1±13.3	0.86		
24-hour ECG	n=57	n=32			
Mean HR (24 hour ECG) (bpm)	74.2±9.6	78.1±11.5	0.10		
Non-sustained VT (%)	33.9	12.5	0.03	0.3	0.1–0.9
SDNN (ms)	101.7±38.1	125.7±53.3	0.09		

BNP: natriuretic brain peptide; CI: confidence interval; HR: heart rate; LA: left atrial; LV: left ventricular; LVVR: left ventricular reverse remodeling; OR: odds ratio; SDNN: standard deviation of NN interval; VT: ventricular tachycardia. Other abbreviations as in Table 1.

A favorable response to drug therapy with ACEI, beta-blockers and aldosterone antagonists was reported, with almost complete reversal of LV dysfunction. An increase in EF of more than 15 units has been described, associated with increases in functional capacity and cardiac index and a decrease in pulmonary capillary pressure, associated with a better prognosis.[11–14] Treatment of HF can influence hemodynamics by decreasing LV afterload and preload. The experimental literature suggests that alterations in the biology and contractility of the failing cardiac myocyte may be reversible after beta blockade. Recent studies in patients treated with beta-blockers who had an increase in EF also showed favorable changes in myocardial gene expression: an increase in sarcoplasmic reticulum calcium ATPase mRNA and alpha-myosin heavy chain mRNA and a decrease in beta-myosin heavy chain mRNA.[15]

In our study, patients with LVRR more often had hypertension and appeared to be at an earlier stage of the disease, with lower LVDD, shorter QRS interval, less LBBB and more favorable ventilatory efficiency. Patients with hypertension and LV dysfunction respond to appropriate afterload-reducing therapy with improvements in LV function, and probably more frequently and more rapidly reach maximum drug titration with beta-blockers and ACEI.

Although only 14% of patients had AF at first consultation, the higher percentage of AF among patients who recovered EF was somewhat surprising. One possible explanation is that AF might have developed simultaneously with heart failure, causing functional changes (irregular and rapid rhythm, loss of atrioventricular synchrony, and loss of atrial transport), which would then show maximum benefit from medical therapy, with reversal of ventricular dysfunction.[16]

The predictors of RRVE in CPET were higher POUE and lower $dVE/VCO_2/VO_2$. Decreased oxygen efficiency slope and lower ventilatory efficiency, determined by the VE/CO_2 slope, additionally normalized for peak VO_2, are sensitive and early prognostic factors of heart failure, reflecting more advanced disease.[17,18]

Our results are consistent with other studies that set out to define the clinical variables associated with improvement in LVEF. Cicoira et al.[19] evaluated 98 patients with idiopathic DCM, and found that those who recovered LV systolic function had shorter duration of symptoms, worse NYHA class and a history of hypertension. In a large study,[20] LVRR was found in 89 of 242 idiopathic DCM patients (37%) and baseline predictors were higher systolic blood pressure and absence of LBBB. Binkley et al.[21] showed that patients who recovered LV function were younger, had higher systolic blood pressure,

Table 3 Predictive factors of left ventricular reverse remodeling on cardiopulmonary exercise testing and cardiac magnetic resonance imaging.

	No LVRR	LVRR	p	OR	CI
CPET	n=41	n=14			
Peak VO_2 (ml/kg/min)	17.6±5.6	19.9±4.9	0.16		
%VO_2 predicted (%)	59.8±17.8	68.4±18.8	0.13		
% VO_2 at AT (%)	39.1±17.1	43.8±13.3	0.38		
VE/CO_2 slope	40.9±14.7	35.3±7.8	0.20		
$VE/VCO_2/VO_2$ peak	4.0±3.4	2.5±1.4	0.05	0.7	0.4–1.0
O_2 pulse (%)	78.9±26.6	84.9±23.0	0.46		
Circulatory power (mmHg/ml/kg/min)	2415.9±866.3	2893.5±914.0	0.09		
POUE	734.0±245.2	979.0±181.6	0.03	1.01	1.0–1.1
POUE at AT	274.9±17.1	327.2±80.9	0.09		
Δ HR recovery at 1 min (bpm)	18.4±8.2	22.9±9.8	0.22		
CMR	n=24	n=14			
EF (%)	30.4±10.1	34.4±9.0	0.24		
Cardiac index (l/min/mm^2)	3.1±0.7	2.9±0.3	0.53		
RV EF (%)	47.9±1.1	52.3±7.8	0.23		
LGE (%)	58.3	50.0	0.74		
LGE >one segment (%)	50.0	42.9	1.0		

AT: anaerobic threshold; CI: confidence interval; CMR: cardiac magnetic resonance; CPET: cardiopulmonary exercise testing; EF: ejection fraction; LGE: late gadolinium enhancement; LV: left ventricular; LVRR: left ventricular reverse remodeling; OR: odds ratio; POUE: peak oxygen uptake efficiency; RV: right ventricular; VCO_2: carbon dioxide output; VE: expired ventilatory flow; VO_2: oxygen uptake.

lower serum creatinine, shorter QRS interval, lower prevalence of diabetes and higher prevalence of hypertension, were more frequently female and had a lower prevalence of ischemic cardiomyopathy.

It has been postulated that non-ischemic etiology has a higher probability of reverse remodeling. This appears to be related to a higher degree of adrenergic activation for an equivalent degree of myocardial dysfunction and to a greater extent of viable myocardium in patients with idiopathic DCM. A marked reduction in sympathetic activity appears to reduce mortality. The extent of heart rate reduction, rather than its baseline level, appears to be associated with a greater increase in LV function.[22]

Contractile reserve has been suggested as a key predictor of LVRR, according to studies with dobutamine echocardiography[23] and positron emission tomography.[24] CMR with gadolinium administration indirectly demonstrates contractile reserve in patients with idiopathic DCM, through the presence of myocardial fibrosis. Some studies have assessed the prognostic value of CMR in non-ischemic DCM. In the study by Assomull et al., [25] midwall fibrosis was present in 35% of 101 patients and was associated with a higher rate of the primary combined endpoint of all-cause death and cardiovascular events. In one study of recent-onset DCM, the lower extent of LGE and the higher edema ratio at CMR were the most important baseline predictors of LVRR.[10] In our study, the presence or extent of LGE was not a predictor of LVRR, possibly due to the small study population.

QRS duration is one of the most sensitive independent predictors of survival in patients with DCM. In our population, mean QRS duration of patients who did not recover LV function was 130 ms. This finding is consistent with recommendations for biventricular pacing. Patients with LVRR also less often had non-sustained ventricular tachycardia on 24-hour ECG, probably also reflecting some positive electrical remodeling.

To summarize, these variables probably discriminate patients in whom EF can recover with medical therapy only from those who may require resynchronization devices or more aggressive strategies, including heart transplantation. Patients whose LV function recovers no longer have indication for ICD or CRT therapy, thus complicating the timing of implantation of these devices. Although current guidelines suggest that an ICD is indicated only in patients already receiving maximal medical therapy, it is not clear how safe it is to wait for optimization of therapy before ICD implantation. We can postulate that in patients with LBBB, low systolic blood pressure and larger LV diameters, it may not be safe to wait for ICD/CRT implantation.

Study limitations

In this study we did not perform the expected number of CMR and CPET exams.

Another study is ongoing in our HF clinic, in a cohort of idiopathic DCM patients, all in sinus rhythm, assessing emerging laboratory predictors of LVRR and obtaining detailed echocardiographic data, with volumetric measures and myocardial deformation changes.

Conclusions

LVRR occurred in approximately one third of patients with idiopathic DCM, and these patients appeared to be at an early stage of the disease, had higher blood pressure and had maximal therapy titration. In these cases there is no longer

indication for ICD or CRT implantation, thus complicating the timing of implantation of these devices.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that they have followed the protocols of their work center on the publication of patient data.

Right to privacy and informed consent. The authors have obtained the written informed consent of the patients or subjects mentioned in the article. The corresponding author is in possession of this document.

Conflicts of interest

The authors have no conflicts of interest to declare.

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2. Left ventricular reverse remodeling in dilated cardiomyopathy: maintained subclinical myocardial systolic and diastolic dysfunction

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ORIGINAL PAPER

Left ventricular reverse remodeling in dilated cardiomyopathy-maintained subclinical myocardial systolic and diastolic dysfunction

Sandra Amorim[1] · João Rodrigues[1] · Manuel Campelo[1] · Brenda Moura[1] · Elisabete Martins[1] · Filipe Macedo[1] · J. Silva-Cardoso[1] · M. Júlia Maciel[1]

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Abstract In idiopathic dilated cardiomyopathy (DCM), myocardial deformational parameters and their relationships remain incompletely characterized. We measured those parameters in patients with DCM, during left ventricular reverse remodeling (LVRR). Prospective study of 50 DCM patients (in sinus rhythm), with left ventricular ejection fraction (EF) <40%. LVRR was defined as an increase of ten units of EF and decrease of diastolic left ventricular diameter (LVDD) in the absence of resynchronization therapy. Performed morphological analysis, myocardial performance quantification (LV and RV Tei indexes) and LV averaged peak systolic longitudinal strain (SSR long) and circumferential strain (SSR circ). At baseline, mean EF was 25.4 ± 9.8%, LVDD was 62.4 ± 7.4 mm, LVDD/BSA of 34.2 ± 4.5 mm/m^2 and 34% had MR grade >II/IV. LVRR occurred in 34% of patients within 17.6 ± 15.6 months and was associated with a reduced rate of death or heart failure hospitalization (5.9% vs. 33.3; p = 0.03). Patients with LVRR had a final EF of 48.9 ± 7.9% (Δ LV EF of 22.4%) and there was a significant decrease (p < 0.05) in: LVDD/BSA, LV systolic diameter/BSA, LV diastolic volume, LV systolic volume, LV mass; an increase (p < 0.05) in sphericity index. However, measures of diastolic function (LA volume/BSA, e′velocity and' E/e′ratio), final LV and RV Tei indexes were not significantly different from baseline. Additionally, final SSR circ and SSR long values were not different from basal. Patients who recovered EF >50% (n = 10), SSR circ and SSR long were inferior to normal. Improvement in EF occurred in one-third of DCM pts and was associated with a decrease of major cardiac events. There was an improvement of diastolic and systolic volumes and in sphericity index, confirming truly LV reverse reshaping. However, myocardial performance indexes, SSR long and SSR circ in reverse-remodeled DCM were still abnormal, suggesting a maintained myocardial systolic and diastolic dysfunction.

Keywords Idiopathic dilated cardiomyopathy · Left ventricular reverse remodeling · Strain rate analysis · Myocardial performance index

Introduction

Progression of heart failure (HF) is associated with left ventricle (LV) remodeling, which manifests as gradual increases in left ventricular end-diastolic and end-systolic volumes, wall thinning, and a change in chamber geometry to a more spherical, less elongated shape, with a continuous decrease in ejection fraction [1]. When ventricular remodeling is advanced, it begins to be self-supporting and capable of conducting the progression of the disease, regardless of neurohormonal status. This explains why medical therapies lose their effectiveness in terminal HF, and some device-based therapies (cardiac resynchronization and mechanical ventricular assistance), that can affect the remodeling of the LV, have been beneficial. Left ventricular reverse remodeling (LVRR) is characterized by decrease of LV dimensions, normalization of LV shape and improvement of systolic function. A favorable response to drug therapy with ACEI, β-blockers and aldosterone antagonists was reported, with almost complete reversal of LV dysfunction. An increase in left ventricular ejection fraction (EF) of more than 15 units has been described, associated

✉ Sandra Amorim
sandra.maria.amorim@netcabo.pt

[1] Cardiology Department, Centro Hospitalar São João, Alameda Prof. Hernâni Monteiro, 4200-319 Porto, Portugal

with an increase in functional capacity, an increase in cardiac index and a decrease in pulmonary capillary pressure, associated with a better prognosis [2–5]. The improvement of myocyte Ca^{2+} handling or the restoration of the response of down-regulated β-adrenergic receptors to sympathetic activation may play a role in normalizing EF in patients with dilated cardiomyopathy (DCM) [6]. Molecular mechanisms of reverse remodeling have not been fully elucidated.

The existence of the new-called HF with recovered ejection fraction (HF-Recovered) represents a distinct HF phenotype with biochemical properties and natural history that differs from the traditional HF population [7]. Predictors of LVRR probably discriminate patients in whom EF can recover only with medical therapy, from patients who may require cardiac devices or referring for heart transplantation.

EF is the most widely used parameter for the global assessment of LV systolic dysfunction. A combined myocardial performance index (isovolumic contraction time plus isovolumic relaxation time divided by ejection time, 'Tei index') has been applied in the echocardiographic evaluation of patients with DCM [8]. This index can also be obtained by tissue Doppler imaging (Tei-TDI) [9].

In recent years, novel technologies, like speckle tracking echocardiography, are useful to detect and comprehend the abnormalities that occur in cardiac diseases. LV global strain is an accurate and sensitive measure of myocardium deformation, allowing the angle-independent quantification of myocardial function in 2D, based on the LV active shortening in the longitudinal, circumferential and radial direction, which is more reproducible than EF and does not rely on geometrical assumptions [10].

There is insufficient research about regional myocardial function and strain rate analysis in patients with normalized EF after optimal pharmacologic therapy. One study demonstrated subclinical LV dysfunction by strain rate analysis at rest and during exercise in patients with normalized EFs [7] and studies on Tei index changes are only described after mechanical LVRR [11, 12]. The aim of this prospective study was to evaluate echocardiographic parameters of patients with idiopathic DCM, comparing the results after optimal pharmacologic therapy, particularly in patients with reverse-remodeled cardiomyopathy.

Methods

Study population

We included consecutive adult patients with DCM followed in a HF outpatient clinic, with a diagnosis of less than 24-month duration and with two initial values of EF of <0.40 more than 1 year apart. This study respects to a recent cohort of patients following a previous published investigation [13], conducted by the same authors.

We excluded patients with ischemic cardiomyopathy: history of myocardial infarction or angina, significant coronary artery disease more than 50% diameter narrowing in any of the major coronary arteries or their branches, positive exercise or pharmacological stress-induced perfusion abnormalities on nuclear scintigraphy or with positive ischemic gadolinium late-enhancement on cardiac magnetic resonance imaging (MRI). We also excluded patients with other secondary forms of DCM: history of moderate or severe hypertension; diabetes mellitus with end-organ damage or on insulin therapy, primary mitral or aortic valvular disease of at least moderate degree; heavy alcohol use (>100 g/day), chemotherapy-induced and peripartum cardiomyopathy. We didn't include patients with acute HF with positive biopsy of active myocarditis, with positive serology for acute phase of bacterial or viral infection or with a cardiac MRI with a suspicion of acute myocarditis. All patients were in sinus rhythm and patients with history of uncontrolled atrial and ventricular arrhythmias were excluded.

At baseline, patients underwent clinical assessment, transthoracic echocardiogram and blood laboratory measurements. Patients were managed according to current clinical practice guidelines [14] and clinicians aimed to reach the recommended target doses for all therapies. During the follow up, periodic clinical evaluation, laboratory measurements and echocardiogram were performed on a 3–6-month basis. This study was performed in accordance with the recommendations set by the Declaration of Helsinki [15] and with the local legal requirements. Our observational study was also performed according to the recommendations of the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement [16].

Definition of LVRR

LVRR was defined by the simultaneous presence of the following conditions: (a) occurrence in two subsequent echocardiograms of an absolute increase of ten units of EF, concomitant with a decrease in diastolic left ventricular diameter (LVDD), without worsening of mitral regurgitation (MR), if present; (b) this increase occurred in the absence of cardiac resynchronization therapy (CRT) or mechanical ventricular assistance. Patients who received CRT were considered to have no LVRR, so we only considered in the analysis the echocardiographic parameters measured before the implantation of CRT.

Transthoracic echocardiography protocol

A standardized complete echocardiographic examination was performed, at baseline and during follow-up, using a commercially available Vivid 7 system (GE Vingmed, Horton, Norway), with a M4S (2.5-MHz) probe. Digital grayscale two-dimensional cine loops from three consecutive heartbeats were obstained from standard apical views (four chamber, two chamber, and long axis) and standard LV short-axis views (basal, mid and apical) at depths of 11 to 20 cm; frame rates were 45–90 Hz.

The chamber quantification parameters were measured according to the professional standards defined by the American Society of Echocardiography and the European Association of Echocardiography [17], EF (%) was calculated by Simpson's biplane method; degree of mitral and tricuspid regurgitation by Doppler, scored on a scale from 0 to 4; pulmonary artery systolic pressure (PASP) was calculated by tricuspid velocities. LV mass was calculated using the formula proposed by Devereux et al. [18]. LV sphericity index was calculated as the ratio of dimensions of long axis view and minor axis view. The early diastolic (E) and atrial (A) wave velocities, the E/A ratio, and the E-wave deceleration time were measured using pulsed wave Doppler recording from the apical four-chamber view. Spectral pulsed-wave Doppler–derived early diastolic velocity (e´) was obtained from the septal and lateral mitral annulus and an average was used. E/e′ratio was calculated to obtain an estimate of LV filling pressure.

The left ventricular global myocardial index (LV Tei-index) determined was calculated as Mitral Valve Closure to Opening Time (MVCO) LV Ejection time/LV Ejection time. It was measured at the septal and lateral sites of the mitral annulus, and the average was utilized. RV myocardial performance index (RV Tei index) was determined as the difference in duration between tricuspid regurgitation and pulmonary ejection divided by pulmonary ejection duration.

Speckle-tracking circumferential strain rates were assessed from basal, mid, and apical LV short-axis views, and the longitudinal strain rate was assessed from the basal, mid, and apical levels in apical four-chamber, two-chamber, and long-axis views. For speckle-tracking strain rate analysis, the peak of the R wave on the electrocardiogram was used as the reference time point for end-diastole. The endocardial border was traced manually in the end-diastolic frame. The software subsequently automatically traced the borders in the other frames. Segments which failed to track were manually adjusted by the operator. Graphical displays of deformation parameters for each segment were then generated automatically. Circumferential and longitudinal global strain was obtained by averaging the peak strain values from the 18 regional longitudinal strain curves: SSR circ and SSR long. Normal values for SSR circ and SSR long were considered −20.9 to −27.8 and −15.9 to −22.1%, respectively, according to literature [19]. All data were stored digitally for off-line analysis on Echo-Pac PC software (7.3.0 GE, Horton Norway) and was performed by two echocardiography specialists, blinded to the study.

Statistical analysis

All values are reported as mean ± SD, median ± interquartile range or as percentages according to characteristics of data. Differences between subjects in each arm were assessed using X^2 test for categorical variables and Student's t-test or Mann–Whitney test for continuous variables, as appropriate. A two-tailed $p < 0.05$ was considered to indicate statistical significance.

To evaluate changes from baseline a paired Student's t-test was used. Data were analysed using SPSS 23.0 statistical package (SPSS Inc., Chicago, IL, USA).

Results

We studied 50 patients, 28 men (56%), aged 59 ± 10 years, followed for 39 ± 22 months. The majority of patients were in NYHA class II (62%). Sixty percent of patients performed coronariography and 78% of patients performed cardiac MRI to rule out ischemic cardiomyopathy or myocarditis.

On EKG, 66% had left bundle branch block (LBBB), 22% had LV hypertrophy criteria and all were in sinus rhythm.

At the end of the follow-up, 94% were treated with angiotensin-converting enzyme inhibitors (ACEI)/ angiotensin II receptor blockers (ARB), 98% with β-blockers, 60% with aldosterone antagonists. Optimal recommended doses of ACEI/ARB were reached in 42% (20–30 mg lisinopril, 5–10 mg perindopril, 16–32 mg candesartan) and optimal doses of β-blockers were reached in 48% (25–50 mg bid carvedilol, 5–10 mg bisoprolol). Only 4% died (2 deaths), 22% were hospitalized for HF worsening and 48% implanted cardiac devices: implantable cardiac defibrillator (ICD) in 40%, CRT plus ICD in 8%.

At baseline, mean left ventricular EF was 25.4 ± 9.8%, LVDD was 62.4 ± 7.4 mm, LVDD/BSA of 34.2 ± 4.5 mm/m^2 and MR grade >II/IV was present in 34% of patients.

Left ventricular reverse remodeling (LVRR)

LVRR occurred in 34% of patients (n = 17) within 17.6 ± 15.6 months of medical therapy. Mean time interval between baseline and final echocardiogram was 38.5 ± 21.9 months. Between groups (LVRR or no LVRR) there was

no difference of mean time of follow-up echocardiograms (43.9 ± 2.4 vs. 35.8 ± 22.0; p = 0.21). The initial LVEF of patients who recovered LV function was 24.9 ± 9.0% and was not different from the value of 26.5 ± 11.2% (p = 0.58) of those who did not recover.

We found that patients who recovered LV function had, at baseline: younger age (54.7 ± 10.8, vs. 60.6 ± 8.8; p = 0.05) and smaller LVDD/BSA (mm/m^2) (32.3 ± 4.8 vs 35.2 ± 4.1, p = 0.03). See Tables 1 and 2 for further details.

Patients that had LVRR had a lower BNP at the end of follow-up (36.9 ± 34.3 vs. 143.5 ± 137.5; p < 0.01) and less major cardiac events (death or HF hospitalization) (5.9% vs. 33.3; p = 0.03), compared to those that didn't have LVRR. In these patients, the heart rate decreased significantly during follow-up (67.6 ± 19.1 vs. 73.9 ± 11.7 bpm, p = 0.02), probably related to the effect with β-blockers. However, LBBB rate and QRS duration didn't change from baseline (58.8% vs. 47.1, p = 0.50; 136.1 ± 34.9 vs. 133.7 ± 35.2, p = 0.66, respectively).

Patients with LVRR had a final EF of 48.9 ± 7.9% (Δ LV EF of 22.4%), and had a significant decrease (p < 0.05) (see Table 3) in: LVDD (53.5 ± 6.7 mm; Δ LVDD of ± 7.2 mm), LVDD/BSA (28.3 ± 3.0 mm/m^2), LV systolic diameter/BSA (20.5 ± 0.6 mm/mm^2), LV

Table 1 Baseline clinical parameters and final therapy of patients without LVRR (LVRR−) and with LVRR (LVRR+)

	LVRR − (n = 33)	LVRR + (n = 17)	p
Age (years)	60.6 ± 8.8	54.7 ± 10.8	0.04
Male sex (%)	54.5	58.8	0.77
BMI	27.4 ± 4.1	29.4 ± 4.4	0.12
Hypertension (%)	57.6	58.8	0.93
Diabetes (%)	24.2	35.3	0.41
CPOD (%)	3.0	11.8	0.22
NYHA class I (%)	27.3	17.6	0.45
NYHA class II (%)	57.6	70.6	0.37
NYHA class III–IV (%)	12.5	11.8	0.94
Heart rate (bpm)	77.7 ± 14.9	73.6 ± 11.3	0.98
Systolic blood pressure (mmHg)	124.5 ± 18.0	123.4 ± 21.7	0.85
QRS duration (ms)	140.6 ± 27.7	136.1 ± 33.8	0.13
LBBB (%)	69.7	58.8	0.44
BNP (g/ml) (median ± IQR)	171.1 ± 530.1	81.3 ± 198.4	0.50
Baseline therapy (%)			
ACEI /ARB	81.8	64.7	0.18
Maximal dose ACEI/ARB	18.2	29.4	0.36
β- Blockers	57.6	70.6	0.37
Maximal dose β-blockers	6.1	5.9	0.98
Aldosterone antagonist	12.1	17.6	0.59
Ivabradin	0.0	5.9	0.98
Diuretics	57.6	47.1	0.48
ICD	0.0	0.0	–
CRT-D	0.0	0.0	–
Final therapy (%)			
ACEI /ARB	93.9	94.1	0.98
Maximal dose ACEI/ARB	42.4	41.2	0.93
β-Blockers	97.0	100	0.47
Maximal dose β-blockers	42.4	58.8	0.27
Aldosterone antagonist	63.6	52.9	0.46
Ivabradin	6.1	17.6	0.20
Diuretics	66.7	64.7	0.89
ICD	45.5	29.4	0.27
CRT-D	12.1	0.0	0.13

ACEI angiotensin-converting enzyme inhibitors, *ARB* angiotensin receptor blockers, *BMI* body mass index, *CPOD* chronic pulmonary obstrutive disease, *LBBB* left bundle branch block, *LVVR* left ventricular reverse remodelling, *NYHA* New York heart association, *RV* right ventricle, *ICD* implantable cardiac defibrillator, *CRT- D* cardiac resynchronization therapy plus ICD

Table 2 Baseline echocardiography parameters of patients without LVRR (LVRR−) and with LVRR (LVRR+)

	LVRR − (n = 33)	LVRR + (n = 17)	p
LV ejection fraction (%)	24.9 ± 9.0	26.5 ± 11.2	0.58
LA volume/BSA (ml/m^2)	70.3 ± 26.3	67.4 ± 25.0	0.47
LVDD (mm)	63.3 ± 7.6	60.7 ± 6.9	0.25
LVDD /BSA (mm/m^2)	35.2 ± 4.1	32.3 ± 4.8	0.03
LV mass/BSA (g/m^2)	167.4 ± 24.7	161.5 ± 35.2	0.49
LV volume/BSA (ml/m2)	111.6 ± 30.0	106.4 ± 27.3	0.57
LV Tei index	0.78 ± 0.34	0.82 ± 0.37	0.15
Mitral regurgitation ≥grade II (%)	36.4	29.4	0.62
PASP (mmHg)	31.0 ± 9.4	29.6 ± 7.8	0.65
RV dimension (mm)	26.5 ± 2.8	28.4 ± 3.6	0.06
RV Tei index	0.46 ± 0.16	0.52 ± 0.32	0.31
RV S velocity (m/s)	0.13 ± 0.02	0.12 ± 0.02	0.56
E/e′	14.7 ± 7.1	11.9 ± 5.2	0.19
E′velocity (m/s)	0.07 ± 0.03	0.07 ± 0.01	0.86
SSR circ (%)	−9.76 ± 11.07	−8.42 ± 2.92	0.66
SSR long (%)	−9.58 ± 3.23	−10.66 ± 4.06	0.36

BSA body surface area, *LV* left ventricle, *LVDD* left ventricular end-diastolic diameter, *LA* left atrial, *LVVR* left ventricular reverse remodeling, *PASP* pulmonary artery systolic pressure, *RV* right ventricle

Table 3 Comparison basal and final echocardiography parameters in patients with LVRR

n = 17	Basal	Final	p
LV ejection fraction (%)	26.5 ± 11.2	48.9 ± 7.9	**< 0.01**
LVDD (mm)	60.7 ± 6.9	53.5 ± 6.7	**0.01**
LVDD/BSA (mm/mm^2)	32.3 ± 4.8	28.3 ± 3.0	**< 0.01**
LV systolic diameter (mm)	54.4 ± 7.9	41.5 ± 4.6	**< 0.01**
LV systolic diameter/BSA (mm/mm^2)	25.9 ± 3.5	20.5 ± 0.6	**0.02**
LV mass (g)	297.2 ± 49.0	233.9 ± 68.8	**0.02**
Sphericity index	1.44 ± 0.22	1.57 ± 0.18	**0.02**
LV diastolic volume (ml)	201.4 ± 48.5	145.5 ± 32.7	**< 0.01**
LV systolic volume (ml)	152.4 ± 55.9	73.6 ± 25.2	**< 0.01**
LV Tei index	0.82 ± 0.38	0.74 ± 0.23	0.45
SSR circunferencial (%)	− 8.48 ± 2.85	− 4.80 ± 4.02	0.31
SSR longitudinal (%)	−10.27 ± 3.77	− 13.06 ± 2.90	0.08
LA volume/BSA (ml/mm^2)	35.6 ± 14.4	28.8 ± 3.8	0.27
E/e′ratio	11.6 ± 4.6	10.47 ± 4.2	0.49
e′velocity (cm/s)	6.7 ± 1.5	7.7 ± 2.2	0.14
RV Tei índex	0.56 ± 0.35	0.39 ± 0.17	0.13

BSA body surface area, *LV* left ventricle, *LVDD* left ventricular end-diastolic diameter, *LA* left atrial, *RV* right ventricle, *SSR* systolic strain rate

diastolic volume (145.5 ± 32.7 ml), LV systolic volume (73.6 ± 25.2 ml), LV mass (233.9 ± 68.8 g); and an increase ($p < 0.05$) in sphericity index (1.57 ± 0.18) and only 5.9 patients (n = 1) had a final MR ≥ rade II/IV.

Controversially, in patients with reverse remodeled DCM, measures of diastolic function as LA volume/BSA, e′velocity and E/e′ratio, were not significantly different from baseline (detailed in Table 3). Also, surprisingly, there weren't significant changes in LV Tei index from baseline (basal: 0.82 ± 0.38; final: 0.74 ± 0.23; $p = 0.45$). This was also true for RV Tei index (basal: 0.56 ± 0.35; final 0.39 ± 0.17; $p = 0.13$). Additionally, final strain values were not significantly different from basal: SSR circ: −8.48 ± 2.85 vs. −4.80 ± 4.02%; $p = 0.31$, SSR long: −10.27 ± 3.77% vs. − 13.06 ± 2.90%; $p = 0.08$.

Comparing patients with LVRR and no LVRR, there weren't significant differences in final LV Tei index (0.75 ± 0.22 vs. 0.85 ± 0.23 p = 0.15), in final SSR circ (−10.0 ± 4.2% vs. −8.2 ± 3.1%, p = 0.17) and in SSR long (−12.5 ± 3.0 vs. 11.5 ± 4.2%, p = 0.40).

In the group of patients who improved EF ≥50% (n = 10), SSR circ was substantially inferior to normal (−10.0 ± 4.1%) and SSR long was also inferior, although close to normal values (−13.1 ± 3.5%). See Table 4 for further details. In Fig. 1 there is an example of a patient with LVRR who improved EF to 56%, but still have a diminished SSR circ and SSR long.

A subgroup analysis in patients with LVRR and hypertension revealed that final SSR circ was significantly lower (−7.68 ± 3.05% vs. −10.74 ± 3.77%; p = 0.01), compared to patients without hypertension. We didn't find differences in another strain rate parameters. The subgroup analysis of basal and final strain rates in patients with diabetes didn't show any significant differences between groups.

Discussion

HF has classically been a clinical syndrome associated with cardiac dilatation and impaired cardiac contractility. Left ventricular EF is the most extensively investigated echocardiographic systolic function parameter and has been established as a powerful predictor of mortality for patients with HF. The myocardial performance index is a Doppler-derived time interval index that combines both systolic and diastolic cardiac performance. The Tei index is easily derived using conventional pulsed Doppler echocardiography, as previously described by Tei and colleagues [8]. The mean normal value of the Tei index is 0.39 ± 0.05 for the LV, while for the right ventricle (RV) it is 0.28 ± 0.04 [8, 20]. Higher index values correspond to more pathological states with overall cardiac dysfunction. The Tei index appears to have close correlation with the widely accepted systolic and diastolic hemodynamic parameters, is a useful method for the study of congestive HF syndrome and has been shown to have strong prognostic value in severe cardiac diseases, such as DCM. A study of Dujardin et al. [21] showed that Tei index and EF were the most significant independent predictors of outcome in patients with DCM. Ikeda et al. [22] demonstrated that patients with DCM and cardiac events had higher LV and RV Tei indexes at the initial follow-up examination; and RV Tei index had a significant linear correlation with LV Tei index. The 6-year survival rate was significantly lower in patients with both LV Tei index ≥ 0.78 and RV Tei index ≥ 0.49 than in other patients [22]. In our study, there was a decrease in RV and LV Tei indexes in patients that had recovery in EF, but didn't reach normal values, indicating that those patients have risk of cardiac events and maintained systolic and diastolic dysfunction.

Experimental and clinical studies showed that LV systolic function is a complex, coordinated action involving longitudinal contraction, circumferential shortening, and radial thickening [23]. Strain rate imaging has a theoretic advantage over Doppler tissue imaging that is relatively immune to cardiac translational motion and tethering [24]. Myocardial strain is comprised by three components:

Table 4 Comparison basal and final echocardiography parameters in patients with LVRR with EF ≥ 50%

n = 10	Basal	Final	p
LV ejection fraction (%)	32.6 ± 8.9	54.5 ± 3.9	**<0.01**
LVDD (mm)	66.4 ± 27.0	27.0 ± 6.8	**<0.01**
LVDD/BSA (mm/mm^2)	31.3 ± 4.4	27.9 ± 2.8	**0.02**
LV systolic diameter (mm)	51.9 ± 8.4	39.3 ± 1.9	**<0.01**
LV systolic diameter/BSA (mm/mm^2)	26.5 ± 11.2	48.9 ± 7.9	**<0.01**
LV mass (g)	308.8 ± 36.5	245.4 ± 61.4	**0.02**
Sphericity index	1.47 ± 0.24	1.55 ± 0.18	0.16
LV diastolic volume (ml)	189.6 ± 46,9	150.7 ± 28.9	**0.02**
LV systolic volume (ml)	129.6 ± 48.1	71.8 ± 21.3	**<0.01**
LV Tei index	0.98 ± 0.38	0.71 ± 0.20	0.05
SSR circunferencial (%)	−9.40 ± 1.96	−9.16 ± 3.55	0.90
SSR longitudinal (%)	−11.24 ± 3.67	−13.15 ± 3.52	0.36
LA volume/BSA (ml/mm^2)	35.7 ± 14.0	28.4 ± 0.8	0.13
E/e′ratio	10.4 ± 3.6	10.1 ± 5.4	0.91
e′velocity (cm/s)	6.50 ± 1.05	7.25 ± 1.94	0.29
RV Tei index	0.61 ± 0.42	0.32 ± 0.15	0.13

BSA body surface area *LV* left ventricle, *LVDD* left ventricular end-diastolic diameter, *LA* left atrial; *RV* right ventricle, *SSR* systolic strain rate

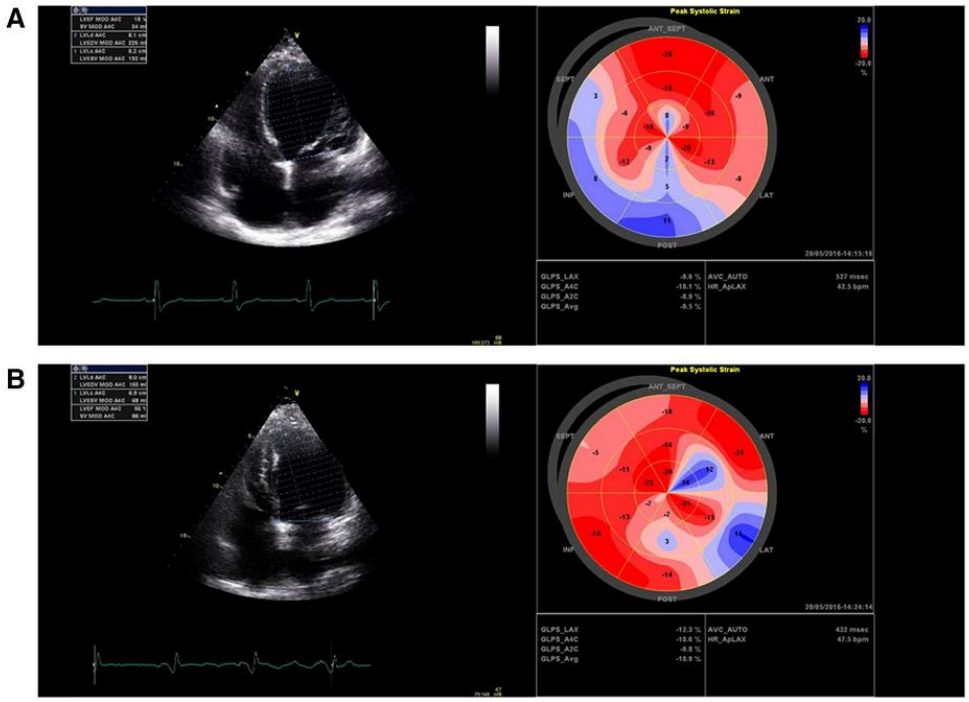

Fig. 1 An example of DCM patient before and after LVRR. **a** Initial LVEF of 15% (calculated by Simpson biplane method) and Bull-eye plot of peak systolic strain, with SScirc of −9.6% and SS long of −9.5%. **b** Final LVEF of 56% and Bull-eye plot of SScirc of −12.3% and SS long of −10.9%

longitudinal, circumferential and radial, disposed in a complex helicoid arrangement, in order to facilitate the ejection and suction of the blood [25]. It is well established that the longitudinal cardiac fibres located in the subendocardium are the first to be affected by myocardial injury [24]. Several authors showed that global strain is a powerful predictor of cardiac events and appears to be a better parameter than EF in patients with HF [26–28]. Circumferential and longitudinal speckle-tracking strain rate analysis can be useful to detect subclinical myocardial systolic and diastolic dysfunction.

In our population, measures of diastolic function, myocardial performance indexes, longitudinal and circumferential strain rate analysis with reverse-remodelled DCM were impaired and were not different from baseline. Additionally, in patients who improved LVEF to normal values, circumferential and longitudinal SSR were still impaired. Thus, multidirectional myocardial analysis may well be important for a better understanding of subclinical myocardial dysfunction in patients with HF. These findings suggest that in treated patients with DCM with reverse remodelling, left ventricular mechanics may not be normal, even when EFs are normal.

Another finding was that in patients with LVRR, LBBB rate and QRS duration didn't change from baseline, and this may contributed to the absence of changes in Tei indexes or global strain parameters. We also found that patients with LVRR and hypertension (although of mild degree) had a lower final SSR circ; this is consistent with other studies that showed that hypertension may contribute to subtle LV dysfunction and affect strain rate parameters [29].

Remains unclear, however, what are the predictors of adverse outcome in patients with reverse-remodelled DCM, defined as depressed left ventricular EF, and normalized after optimal pharmacologic therapy. One study showed that LVRR was a favourable prognostic indicator in patients with DCM irrespective of its detection timing (early vs. late >24 months recovery) [30]. The Penn Heart Failure Study [31], which included of 1821 chronic HF patients divided in three categories based on echocardiograms: HF-reduced EF (HF-REF) if EF was <50%, HF preserved EF (HF-PEF) if EF was consistently ≥50%, and HF-Recovered if EF on enrolment in PHFS was ≥50%, but prior EF was <50%; showed that HF-Recovered is associated with a better event-free survival than HF-REF and HF-PEF. However, these patients continued to experience a significant

number of HF hospitalizations, suggesting persistent HF risk. These authors demonstrated that HF-Recovered patients had abnormal BNP, uric acid, ST2, and sFlt-1 and nearly half had detectable troponin I, indicating that there is persistent neurohormonal activation, increased oxidative stress, and cardiomyocyte injury and stress, despite apparent recovery of EF. These findings provide a rationale to continue background medical or device therapy for HF-Recovered patients. The recurrence was significantly correlated with the discontinuation of heart failure drugs [32]. These results suggest that continuous medical therapy may be mandatory in patients who recover from LV systolic dysfunction.

Study limitations

This study englobes a small number of patients at a single center, so future studies of larger populations may elucidate findings of subclinical systolic and diastolic dysfunction in patients with LVRR. We didn't perform radial strain or LV torsion due to software limitations; those parameters may be important for the comprehension of the mechanism of reverse remodeling in DCM patients.

Conclusions

Improvement in EF occurred in 34% of DCM pts and was associated with better capacity, lower BNP, a decrease in diastolic and systolic volumes and in sphericity index, confirming truly LV reverse reshaping. However, more sensitive measures like myocardial performance and tissue deformational indexes did not show significant changes.

Therefore, measurements of both regional myocardial systolic and diastolic function as assessed by circumferential and longitudinal speckle-tracking strain rates may be very helpful for understanding subtle LV myocardial dysfunction that cannot be detected by conventional echocardiographic parameters such as EF in patients with reverse-remodeled DCM.

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Compliance with ethical standards

Conflict of interest None.

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3. Role of biomarkers in dilated cardiomyopathy- evaluation of clinical severity and reverse remodeling

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ORIGINAL ARTICLE

The role of biomarkers in dilated cardiomyopathy: Assessment of clinical severity and reverse remodeling

Sandra Amorim[a,c,*], Manuel Campelo[a,c], Brenda Moura[a], Elisabete Martins[a,c], João Rodrigues[a], Isaac Barroso[b], Margarida Faria[b], João Tiago Guimarães[b,c,d], Filipe Macedo[a,c], José Silva-Cardoso[a,c], Maria Júlia Maciel[a,c]

[a] *Cardiology Department, Centro Hospitalar São João, Porto, Portugal*
[b] *Clinical Pathology Department, Centro Hospitalar São João, Porto, Portugal*
[c] *Porto Medical School, Porto, Portugal*
[d] *EPIUnit- ISPUP, Institute of Public Health, University of Porto, Porto, Portugal*

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Biomarkers;
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Abstract
Introduction: Biomarkers in dilated cardiomyopathy (DCM) reflect various pathobiological processes, including neurohormonal activation, oxidative stress, matrix remodeling, myocyte injury and myocyte stretch. We assessed the role of biomarkers in clinical and echocardiographic parameters and in left ventricular (LV) reverse remodeling (LVRR).
Methods: In this prospective study of 50 DCM patients (28 men, aged 59±10 years) with LV ejection fraction (LVEF) <40%, LVRR was defined as an increase of >10 U in LVEF after optimal medical therapy.
Results: Baseline LVEF was 25.4±9.8% and LV end-diastolic diameter (LVEDD)/body surface area (BSA) was 34.2±4.5 mm/m^2. LVRR occurred in 34% of patients within 17.6±15.6 months. No correlation was found between B-type natriuretic peptide (BNP), 25-hydroxyvitamin D (25(OH)D), CA-125, high-sensitivity C-reactive protein (hs-CRP), lipoprotein(a) [Lp(a)], noradrenaline, adrenaline, renin or aldosterone and LVRR. Patients in NYHA class III or IV, with pulmonary congestion or ankle edema, had higher CA-125, cystatin C, BNP and hs-CRP levels ($p<0.05$). CA-125 was correlated with BNP ($r=0.61$), hs-CRP ($r=0.56$) and uric acid ($r=0.52$) (all $p=0.01$). BNP correlated directly with LVEDD ($r=0.49$), LV volumes ($r=0.51$), pulmonary artery systolic pressure (PASP) ($r=0.43$) and E/e′ ($r=0.31$), and was inversely correlated with LVEF ($r=-0.50$) and e′ velocity ($r=-0.32$) ($p<0.05$). CA-125 was positively correlated with left atrial volume/BSA ($r=0.46$), E/A ratio ($r=0.60$) and PASP ($r=0.49$) ($p<0.05$).

* Corresponding author.
E-mail address: sandra.maria.amorim@netcabo.pt (S. Amorim).

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Conclusions: No correlation was found between biomarkers and LVRR, but CA-125, BNP and hs-CRP were predictors of clinical severity and congestion. BNP correlated with parameters of systolic and diastolic dysfunction, while CA-125 correlated with measures of diastolic dysfunction.

PALAVRAS-CHAVE
Biomarcadores;
Miocardiopatia dilatada;
Remodelagem reversa

O papel dos biomarcadores na miocardiopatia dilatada – avaliação de gravidade clínica e remodelagem reversa

Resumo
Introdução: Os biomarcadores na miocardiopatia dilatada (DCM) refletem vários processos fisiopatológicos: ativação neuro-hormonal, stresse oxidativo, remodelagem da matriz extracelular, lesão e estiramento miocitários. Procurámos associar biomarcadores com parâmetros clínicos, ecocardiográficos e com a reversão da remodelagem do ventrículo esquerdo (LVRR).
Métodos: Estudo prospetivo de 50 doentes com DCM (28 homens, idade 59±10 anos) com fração de ejeção ventricular esquerda (LVEF) <40%. A LVRR definiu-se como aumento>10 U da LVEF, após a terapêutica médica otimizada.
Resultados: A LVEF basal foi de 25,4±9,8% e o diâmetro do VE (LVD)/BSA de 34,2±4,5 mm/m^2. A LVRR ocorreu em 34%, em 17,6±15,6 meses. Não houve correlação entre BNP, 25-OH-vit D, CA 125, hsCRP, Lp(a), noradrenalina, adrenalina, renina, aldosterona e LVRR. Doentes em classe NYHA (III-IV), com congestão pulmonar ou edema periférico apresentaram níveis mais elevados de CA 125, cistatina C, BNP e hsCRP (p<0,05). O CA 125 correlacionou-se com níveis de BNP (r=0,61), hsCRP (r=0,56) e ácido úrico (r=0,52) (p=0,01). O BNP relacionou-se diretamente com LVD (r=0,49), volume VE (r=0,51), PSAP (r=0,43), razão E/e′ (r=0,31); e inversamente com LVEF (r=-0,50) e vel. e′ (r=-0,32) (p<0,05). O CA 125 correlacionou-se com o volume AE/BSA (r=0,46), razão E/A (r=0,60) e PSAP (r=0,49) (p<0,05).
Conclusões: Não houve correlação entre biomarcadores e LVRR, contudo, o CA125, BNP e hsCRP foram preditores de gravidade clínica e de congestão. O BNP relacionou-se com parâmetros de disfunção sistólica e diastólica, enquanto o CA 125 se relacionou com medidas de disfunção diastólica.

Introduction

Heart failure (HF) is a major public health burden and is often a clinically silent process, with progressive cardiac remodeling that eventually leads to symptomatic presentation late in the course of disease progression. The severity and prognosis of HF vary substantially, ranging from mild disease that is easily managed with neurohormonal blockade to advanced illness requiring mechanical support or heart transplantation.[1] Physicians use biomarkers as additional tools to aid clinical diagnosis and treatment and to identify high-risk subjects.[2]

The progression of HF is complex and is driven by multiple biological processes, including inflammation, oxidative stress, neurohormonal activation, vascular remodeling, myocyte injury, and renal impairment.[3] Current guidelines recommend testing B-type natriuretic peptide (BNP) or N-terminal pro-BNP (NT-proBNP).[4]

The progression of HF is associated with left ventricular (LV) remodeling, which manifests as gradual increases in LV end-diastolic and end-systolic volumes, wall thinning, and a change in chamber geometry to a more spherical, less elliptical shape, with a continuous decrease in LV ejection fraction (LVEF).[5] When ventricular remodeling is advanced, it is self-sustaining, leading to disease progression, regardless of neurohormonal status.

However, in some situations, there may be LV reverse remodeling (LVRR), characterized by decreases in LV dimensions, normalization of LV shape and improvement of systolic function.

In this work, we set out to find associations between biomarkers and clinical severity and echocardiographic parameters. We also sought predictors of LVRR after optimal pharmacological therapy. We used available biomarkers that reflect diverse biological pathways in HF: adrenaline, noradrenaline, plasma renin, aldosterone and BNP (neurohormonal activation), high-sensitivity C-reactive protein (hs-CRP), cancer antigen CA-125 (inflammation), uric acid and lipoprotein(a) [Lp(a)] (oxidative stress), creatinine and cystatin C (renal function), and 25-hydroxyvitamin D

[25(OH)D] (extracellular remodeling), in a cohort of HF outpatients.

Methods

We included consecutive adult patients with dilated cardiomyopathy (DCM) followed in an HF outpatient clinic in a tertiary care center, diagnosed less than 24 months previously and with two values of LVEF of <40% more than one year apart. This is a biomarker substudy of a previously published work[6] by the same authors. In this new cohort we excluded patients with atrial fibrillation, in order to avoid measurement errors in the assessment of LVEF. We also excluded DCM patients with secondary etiologies including ischemic, valvular, inflammatory and toxic cardiomyopathy.

At baseline, patients underwent clinical assessment of symptoms and signs of HF. Peripheral congestion (ankle edema) and pulmonary congestion were assessed, the latter by lung auscultation and chest X-ray. All patients underwent an electrocardiogram (ECG) and transthoracic echocardiogram and blood laboratory tests. Patients were managed according to current clinical practice guidelines[4] and efforts were made to reach the recommended target doses for all therapies. During follow-up, periodic clinical assessment, laboratory tests and echocardiography were performed at three and six months. All patients gave their written informed consent. This study was performed in accordance with the recommendations of the Declaration of Helsinki[7] and approved by the hospital's ethics committee.

Definition of left ventricular reverse remodeling

LVRR was assessed once optimal medical therapy was reached and was defined as the occurrence in two subsequent echocardiograms, more than six months apart, of an absolute increase of 10 units of LVEF, together with a decrease in LV end-diastolic diameter (LVEDD), without worsening of mitral regurgitation, if present, in the absence of cardiac resynchronization therapy (CRT) or mechanical ventricular assistance. Patients who received CRT were considered not to have LVRR, so only echocardiographic parameters measured before implantation of a CRT device were considered in the analysis.

Transthoracic echocardiography protocol

Transthoracic echocardiography was performed at baseline and during follow-up using a commercially available echocardiographic system (General Electric Vivid 7.0) with a 2.5 MHz transducer.

Chamber quantification parameters were measured according to the standards defined by the American Society of Echocardiography and the European Association of Echocardiography.[8] LVEF was calculated by Simpson's biplane method; mitral and tricuspid regurgitation was measured by Doppler, scored on a scale from 0 to 4; and pulmonary artery systolic pressure (PASP) was calculated from tricuspid velocities. LV mass was calculated using the formula proposed by Devereux et al.[9] The LV sphericity index was calculated as the ratio of dimensions in long-axis and short-axis views. Early diastolic (E) and atrial (A) wave velocities, E/A ratio, and E-wave deceleration time were measured using pulsed wave Doppler recording from apical 4-chamber view. Spectral pulsed-wave Doppler-derived early diastolic velocity (e′) was obtained from the septal mitral annulus, and the E/e′ ratio was calculated to obtain an estimate of LV filling pressure.

All data were stored digitally, and off-line data analysis was performed. To assess variability in interpretation, all echocardiograms were analyzed independently by two specialists in echocardiography, blinded to the study. Reproducibility of the measurements was calculated on the basis of standard error of the estimate; both inter- and intraobserver variation were ≤5% for follow-up LVEF.

Biomarker assessment

All biomarkers were measured from plasma obtained at the time of study entry.

Blood samples were collected after a 30-min rest through a venous catheter and the first sample was rejected. Plasma catecholamines were determined by high-performance liquid chromatography (Gilson). BNP was measured by chemiluminescent immunoassay (Abbott). Plasma renin activity and aldosterone was determined by radioimmunoassay. hs-CRP was measured by nephelometry (Vista, Siemens). CA-125 and 25(OH)D were determined by chemiluminescence (Abbott and Roche, respectively).

Statistical analysis

All values are reported as mean ± SD, median ± interquartile range, or percentages, according to the characteristics of the data. Differences between subjects in each arm were assessed using the chi-square test for categorical variables and the Student's t test or Mann-Whitney test for continuous variables, as appropriate. A two-tailed $p<0.05$ was considered to indicate statistical significance.

A paired Student's t test was used to assess changes from baseline. The relationships between biomarkers and echocardiography parameters were analyzed by Spearman's correlation test. Data were analyzed using the SPSS 23.0 statistical package (IBM SPSS Inc., Chicago, IL, USA).

Results

We studied 50 patients, 28 men (56%), aged 59±10 years and followed for 39±22 months. The majority of patients (62%) were in New York Heart Association (NYHA) class II. At baseline, mean LVEF was 25.4±9.8%, LVEDD was 62.4±7.4 mm, LVEDD/body surface area (BSA) was 34.2±4.5 mm/m^2 and grade >II/IV mitral regurgitation was present in 34% of patients. Table 1 details the patients' baseline characteristics.

On the ECG, 66% had left bundle branch block (LBBB), 22% had LV hypertrophy criteria and all were in sinus rhythm.

At the beginning of the study, 76% of patients were being treated with angiotensin-converting enzyme inhibitors (ACEIs)/angiotensin receptor blockers (ARBs), 62% with

Table 1 Baseline characteristics of the study population (n=50).

Age (years)	58.6±9.7
Male (%)	56.0
BMI (kg/m²)	28.1±4.3
Hypertension (%)	58.0
Diabetes (%)	28.0
COPD (%)	6.0
Heart rate (bpm)	76.3±13.8
SBP (mmHg)	124.1±19.1
NYHA class I (%)	24.0
NYHA class II (%)	62.0
NYHA class III-IV (%)	14.0
LBBB (%)	66.0
QRS duration (ms)	139.1±29.7
Therapy (%)	
ACEI/ARB	76.0
Maximal dose ACEI/ARB	22.0
Beta-blocker	62.0
Maximal dose beta-blocker	6.0
Aldosterone antagonist	14.0
Ivabradine	2.0
Diuretic	54.0
ICD/CRT-D	0.0
Echocardiography	
LVEDD (mm)	62.4±7.4
LVEDD/BSA (mm/m²)	34.2±4.5
LVEF (%)	25.4±9.8
LV mass/BSA (g/m²)	165.3±28.5
Mitral regurgitation >grade II/IV (%)	34.0
Sphericity index	1.43±0.21
LVEDV/BSA (ml/m²)	109.7±28.8
LV Tei index	0.79±0.35
LA volume/BSA (ml/m²)	37.3±12.3
E/e′	13.7±6.6
RV dimension (mm)	27.2±3.2
RV S velocity on TDI (m/s)	0.12±0.02
RV Tei index	0.49±0.23

ACEI: angiotensin-converting enzyme inhibitor; ARB: angiotensin receptor blocker; BMI: body mass index; BSA: body surface area; COPD: chronic obstructive pulmonary disease; ICD/CRT-D: implantable cardioverter-defibrillator/cardiac resynchronization therapy device; LA: left atrial; LBBB: left bundle branch block; LV: left ventricular; LVEDD: left ventricular end-diastolic diameter; LVEDV: left ventricular end-diastolic volume; LVEF: left ventricular ejection fraction; NYHA: New York Heart Association; RV: right ventricular; SBP: systolic blood pressure; TDI: tissue Doppler imaging.

beta-blockers and 14% with aldosterone receptor antagonists (Table 1).

At the end of follow-up, 94% of the patients were being treated with ACEIs/ARBs, 98% with beta-blockers and 60% with aldosterone antagonists. Optimal recommended doses of ACEIs/ARBs were reached in 42% and optimal doses of beta-blockers were reached in 48%. Only 4% needed urgent transplantation or died (two deaths), 22% were hospitalized for worsening HF and 40% received an implantable cardioverter-defibrillator (ICD). At the end of follow-up only 15 patients had LBBB and LVEF ≤35%. Four (8%) received a cardiac resynchronization therapy device (CRT-D); the others did not receive a CRT-D because of NYHA class I, technical problems, patient refusal, transient LVEF recovery, presence of LV diverticulum or old age.

Prevalence and prognostic value of left ventricular reverse remodeling

LVRR occurred in 34% of patients within 17.6±15.6 months (mean time to first echocardiogram to show LV recovery). Patients with LVRR had a final LVEF of 48.9±7.9% (Δ LVEF of 22.4%), and had a significant decrease ($p<0.05$) in LVEDD (53.5±6.7 mm; Δ LVEDD of ±-7.2 mm), LVEDD/BSA (28.3±3.0 mm/m²), LV end-systolic diameter (LVESD)/BSA (20.5±0.6 mm/m²), LV diastolic volume (145.5±32.7 ml), LV systolic volume (73.6±25.2 ml), LV mass (233.9±68.8 g); and an increase ($p<0.05$) in sphericity index (1.57±0.18), and only 5.9 patients (n=1) had final ≥grade II/IV mitral regurgitation.

The initial LVEF of patients who recovered LV function was 24.9±9.0% and was not significantly different (p=0.58) from the 26.5±11.2% of those who did not recover.

Patients with LVRR had lower BNP at the end of follow-up (143.5±137.5 vs. 36.9±34.3 pg/ml, $p<0.01$) and fewer major cardiac events (death or HF hospitalization) (5.9% vs. 33.3; p=0.03) compared to those who did not have LVRR.

Predictors of left ventricular reverse remodeling

Baseline predictors of LVRR are detailed in Table 2. Patients who recovered LV function were younger (60.6±8.8 vs. 54.7±10.8 years, p=0.04) and had a lower LVEDD/BSA ratio (35.2±4.1 vs. 32.3±4.8 mm/m²), p=0.03) and higher creatinine clearance (94.3±27.3 vs. 121.3±58.2, p=0.03). No association was found between levels of 25(OH)D, CA-125, hs-CRP, Lp(a), noradrenaline, adrenaline, renin or aldosterone and reverse remodeling.

Biomarkers at clinical presentation

Patients who presented at baseline with worse NYHA class (III/IV) had higher levels of CA-125 (21.6±58.1 vs. 116.0±255.3 U/ml), cystatin C (0.76±0.16 vs. 0.92±0.05 mg/l), BNP (257.5±391.0 vs. 968.5±950.1 pg/ml) and hs-CRP (2.6±3.4 vs. 10.9±13.4 mg/l) ($p<0.05$) (Table 3).

Patients with pulmonary congestion or ankle edema also had higher levels of CA-125, BNP and hs-CRP ($p<0.01$) (Table 3).

Correlations between biomarkers and with echocardiographic parameters

There was a significant correlation between four biomarkers: CA-125, BNP, hs-CRP and uric acid. BNP also correlated negatively with 25(OH)D (r=-0.43, $p<0.05$) (Table 4).

We found some correlations between biomarkers and echocardiographic variables (Table 5). BNP correlated directly with LV dimensions (r=0.49), LV volumes (r=0.51), PASP (r=0.43) and measures of diastolic dysfunction (E/e′)

Table 2 Predictors of left ventricular reverse remodeling.

	No LVRR (n=33)	LVRR (n=17)	p
Age (years)	60.6±8.8	54.7±10.8	0.04
Male (%)	54.5	58.0	0.77
Hypertension (%)	57.6	58.8	0.93
NYHA class I (%)	27.3	17.6	0.99
Heart rate (bpm)	77.7±14.9	73.6±11.3	0.98
SBP (mmHg)	124.5±18.0	123.4±21.7	0.85
QRS duration (ms)	140.6±27.7	136.1±33.8	0.13
LBBB (%)	69.7	58.8	0.44
Laboratory variables			
Hematocrit (%)	41.3±4.4	41.4±4.2	0.98
CrCl (ml/min)	94.3±27.3	121.3±58.2	0.03
Uric acid (mg/dl)	6.5±1.7	6.1±1.9	0.48
Na+ (mEq/l)	139.2±2.5	138.2±2.2	0.38
BNP (g/ml) (median)	171.1±530.1	81.3±198.4	0.50
Adrenaline (pg/ml)	46.2±38.6	29.9±16.9	0.06
Noradrenaline (pg/ml)	519.2±334.6	437.6±195.1	0.38
Renin (U/l)	228.5±446.7	84.9±90.9	0.12
Aldosterone (mg/dl)	10.1±13.7	9.5±9.3	0.89
CA-125 (U/ml)	32.7±76.6	47.8±152.1	0.65
hs-CRP (mg/l)	4.3±6.4	3.7±7.3	0.75
Lp(a) (mg/dl)	37.9±45.0	27.3±32.6	0.58
25(OH)D (ng/ml)	17.3±9.3	19.9±11.1	0.50
Cystatin C (mg/l)	0.78±0.14	0.78±0.19	0.84
Echocardiogram			
LVEF (%)	24.9±9.0	26.5±11.2	0.58
LA volume/BSA (ml/m^2)	70.3±26.3	67.4±25.0	0.47
LVEDD (mm)	63.3±7.6	60.7±6.9	0.25
LVEDD/BSA (mm/m^2)	35.2±4.1	32.3±4.8	0.03
LV mass/BSA (g/m^2)	167.4±24.7	161.5±35.2	0.49
LVEDV/BSA (ml/m^2)	111.6±30.0	106.4±27.3	0.57
LV Tei index	0.78±0.34	0.82±0.37	0.72
Mitral regurgitation ≥grade II (%)	36.4	29.4	0.62
PASP (mmHg)	31.0±9.4	29.6±7.8	0.65
RV dimension (mm)	26.5±2.8	28.4±3.6	0.06
RV Tei index	0.46±0.16	0.52±0.32	0.42
RV S velocity (m/s)	0.13±0.02	0.12±0.02	0.56
E/e′	14.7±7.1	11.9±5.2	0.19
E′ velocity (m/s)	0.07±0.03	0.07±0.01	0.86

25(OH)D: 25-hydroxyvitamin D; BNP: natriuretic brain peptide; CrCl: creatinine clearance; hs-CRP: high-sensitivity C-reactive protein; LA: left atrial; Lp(a): lipoprotein(a); LVEDV: left ventricular end-diastolic volume; LVEF: left ventricular ejection fraction; LVVR: left ventricular reverse remodeling; PASP: pulmonary artery systolic pressure. Other abbreviations as in Table 1.

and was inversely correlated with LVEF (r=-0.50) and e′ velocities (r=-0.32) (p<0.05).

CA-125 correlated with LA volume/BSA (r=0.46), E/A ratio (r=0.60) and PASP (r=0.49) (<0.05).

There was a marginal correlation between hs-CRP and LVEF (r=-0.28, p=0.05), and LV diastolic volume/BSA (r=0.32, p=0.05).

Levels of 25(OH)D were only correlated with PASP (r=-0.42) and E/A ratio (r=0.20) (p<0.05).

Discussion

One of the most important areas of biomarker research is the role of biomarker profiling to better characterize the phenotype of patients who might best respond to therapeutic interventions, whether drug or device therapies.[10] The newly-defined HF with recovered LVEF represents a distinct HF phenotype with biochemical properties and natural history that differ from the traditional HF population.[11] Predictors of LVRR probably distinguish patients in whom LVEF may recover with medical therapy only from patients who may require cardiac devices or more aggressive strategies, including heart transplantation. Patients who have recovered LVEF do not, in theory, have indications for ICD or CRT therapy, thus complicating the timing of implantation of these devices.

Data now suggest that biomarkers may also be useful to predict or monitor LVRR. A new clinical score, the ST2-R2

Table 3 Biomarkers at clinical presentation.

	NYHA I-II n=43	NYHA III-IV n=7	p	No congestion n=37	Congestion n=13	p
CrCl (ml/min)	107.8±43.6	76.9±11.3	0.05	104.9±36.7	99.2±56.0	0.23
Cystatin C (mg/l)	0.76±0.16	0.92±0.04	0.01	0.75±0.14	0.89±0.16	0.28
Uric acid (mg/dl)	6.1±1.6	7.4±2.4	0.10	6.1±1.5	7.0±2.2	0.18
Na+ (mEq/l)	139.0±2.5	138.4±1.9	0.39	138.8±2.4	139.2±2.5	0.96
BNP (g/ml) (median)	102.3±210.4	960.8±2193.0	<0.01	82.1±176.7	464.8±1406.7	<0.01
Adrenaline (pg/ml)	41.5±39.1	34.6±21.8	0.51	44.6±36.8	29.4±17.6	0.07
Noradrenaline (pg/ml)	498.9±311.7	443.1±163.4	0.44	486.3±330.7	501.0±157.4	0.25
Renin (U/l)	149.0±308.1	306.6±580.7	0.09	145.8±329.0	248.6±445.2	0.38
Aldosterone (mg/dl)	10.2±12.9	8.2±7.3	0.56	8.4±7.3	14.4±20.0	0.02
CA-125 (U/ml)	21.6±58.1	132.7±237.2	<0.01	11.5±10.5	115.8±200.4	<0.01
hs-CRP (mg/l)	2.6±3.4	12.6±13.0	<0.01	2.3±2.8	9.4±11.3	<0.01
Lp(a) (mg/dl)	36.2±43.8	25.0±18.4	0.37	41.6±47.3	18.5±12.6	0.04
25(OH)D (ng/ml)	18.8±9.7	9.0±8.5	0.69	20.4±9.8	10.9±5.5	0.13
Iron	97.3±41.5	84.0±32.4	0.88	98.7±43.2	84.1±26.8	0.46

25(OH)D: 25-hydroxyvitamin D; BNP: natriuretic brain peptide; Congestion: pulmonary congestion or ankle edema; CrCl: creatinine clearance; hs-CRP: high-sensitivity C-reactive protein; Lp(a): lipoprotein(a).

Table 4 Correlations between biomarkers.

r (Pearson's correlation)	CA-125	hs-CRP	BNP	25(OH)D
BNP	0.61*	0.50*	–	-0.42*
hs-CRP	0.56*	–	0.50*	-0.16
Uric acid	0.52*	0.56*	0.50*	-0.38

BNP: natriuretic brain peptide; hs-CRP: high-sensitivity C-reactive protein.
* Significant at <0.05.

Table 5 Correlations between biomarkers and echocardiographic parameters.

r (Pearson's correlation)	CA-125	hs-CRP	BNP	25(OH)D
LV diameter	0.32	0.14	0.40*	-0.20
LV diameter/BSA	0.12	0.08	0.44*	-0.23
LVEDV	0.49*	0.33	0.43*	-0.03
LVEDV/BSA	0.23	0.32	0.51*	-0.08
LVEF	-0.22	-0.28	-0.50*	0.30
E/A	0.60*	0.24	0.20	0.47*
PASP	0.49*	0.23	0.43*	-0.42*
LA volume/BSA	0.46*	0.13	0.26	0.10
E/e′	0.09	-0.06	0.31*	0.01
e′	-0.11	0.08	-0.32*	0.21
Sphericity index	-0.18	0.06	-0.27	0.18

* Significant at <0.05; p=0.05.
Abbreviations as in Tables 1 and 2.

score, contains five clinical variables (non-ischemic etiology, absence of LBBB, HF duration, LVEF and beta-blocker treatment) and a remodeling biomarker, soluble toll-like receptor 2 (ST2). A significant relationship was observed between ST2-R2 scores and changes in LVEF, indexed LV sizes, and percentage reduction in LV end-systolic volume index; a similar trend was observed with diastolic parameters.[11]

Our study set out to find predictors of LVRR. In our population LVRR occurred in one third of DCM patients, who were younger and had better renal function and smaller LVEDD. These results are consistent with other studies. In a large study, LVRR was found in 89 of 242 idiopathic DCM patients (37%) and baseline predictors were higher systolic blood pressure and the absence of LBBB.[12] Binkley et al. showed that patients who recovered LV function were younger, had higher systolic blood pressure, lower serum creatinine level, shorter QRS interval, a lower prevalence of diabetes and history of hypertension, were more frequently female and had a lower prevalence of ischemic cardiomyopathy.[13]

In our population LVRR was also associated with lower BNP at the end of follow-up and with favorable outcome and reduced rate of cardiac events. In a recent study with elderly HF patients, intensified medical therapy led to improvement in LVEF and to reverse remodeling. NT-proBNP guided therapy was associated with a greater improvement in LVEF than symptom-guided therapy in both patients aged 60 to 74 and in those aged ≥75 years.[14]

We found no association between BNP, 25(OH)D, CA-125, hs-CRP or Lp(a) and LV reverse remodeling. As expected, patients with poorer functional class, pulmonary congestion, or ankle edema had higher BNP levels. Atrial natriuretic peptide and BNP are produced in response to myocardial stretch due to pressure or volume overload.[15] In the ICON study, increasing severity of HF, as measured by NYHA functional class[16] and symptom severity, correlated directly with increasing BNP concentrations.[17] In our study, BNP also correlated with LV dimensions, LV volumes, LVEF, PASP and measures of diastolic dysfunction (E/e′ and e′ velocities). Since BNP is primarily synthesized by cardiomyocytes, it is not surprising that the highest levels are secreted by the LV. Studies have demonstrated that BNP and NT-proBNP levels correlate positively with LV dimensions, volumes, and mass and are inversely related to LVEF; the strongest correlations have been reported for BNP with LV diastolic wall stress consistent with stretch-mediated BNP secretion.[18,19] BNP levels increase with greater severity

of overall diastolic dysfunction, correlating with indices of filling pressure as well as with indices of compliance and myocardial relaxation.[20,21]

Serum CA-125, a high molecular weight glycoprotein, is a tumor marker widely used in patients with ovarian cancer.[22] Recently, increased serum CA-125 levels, in parallel with catecholamines and natriuretic peptides, have also been documented in patients with HF.[23] However, little is known about the biologic role of this substance: whether it simply reflects increased activation of the cytokine pathway or is an active substance actually responsible for myocardial and/or peripheral dysfunction. In our population, CA-125 was not a predictor of LVRR, but did predict more severe presentation as shown by worse functional class and pulmonary and peripheral congestion. It also correlated with measures of diastolic dysfunction (LA volume, E/A ratio and PASP), as well as with BNP levels, hs-CRP and uric acid, suggesting a potential pathogenic link between inflammatory activation and this marker of systemic congestion. Kouris et al. showed that patients in NYHA classes III and IV had significantly higher mean CA-125 values than patients in class II; serum CA125 levels correlated weakly with PASP and renal function.[24] A study analyzing CA-125 levels and LV dysfunction in patients on hemodialysis showed they were positively correlated with pro-BNP and C-reactive protein (CRP) levels, as well as with LVEDD, LVESD and LV mass index.[25]

CRP is a plasma protein that participates in the systemic response to inflammation, an important mechanism in the progression of HF. A landmark review showed that CRP concentrations were higher than normal in 70% of HF patients, and were directly related to severity of HF.[26] In a recent study of patients with acute HF, both hs-CRP and NT-proBNP were independent predictors of 12-month mortality.[27] In our patients, hs-CRP was associated with clinical severity and marginally correlated with measures of systolic dysfunction (LVEF and LVEDV/BSA). In a previous study, CRP levels increased in parallel with NYHA class and were also related to higher readmission and mortality rates.[28]

Functional vitamin D receptors are present in cardiac cells and their activation affects gene expression, proliferation and contraction of cardiomyocytes. Vitamin D may thus contribute to the development of cardiac hypertrophy and fibrosis.[29] In our population, 25(OH)D was not a predictor of LVRR and was only correlated with PASP and E/A. In a study of subjects with HF, LVEDD, LVESD, LVEDV and LVESV were significantly larger and fractional shortening was lower in patients with 25(OH)D <25 nmol/l than with 25(OH)D ≥25 nmol/l ($p<0.05$); log values of 25(OH)D were negatively correlated with LVEDD and LVEDV ($r=-0.28$; $p<0.05$).[29]

In a recent study of ambulatory patients with chronic HF, Ky et al.[30] tested the hypothesis that a group of seven biomarkers (BNP, soluble fms-like tyrosine kinase receptor, hs-CRP, ST2, cardiac troponin I, uric acid and creatinine) could be combined into a multimarker score that would predict risk of adverse outcome, defined as death, cardiac transplantation or ventricular assist device placement. Patients in the highest tertile of the multimarker score had a 13.7-fold increased risk of adverse outcomes compared with the lowest tertile.

The approximate cost per patient of the biomarkers that we used is currently around €100. In-hospital care is responsible for ~60% of HF-related costs and median hospitalization cost is €9475,[4] but in many cases costs are much higher due to frequent comorbidities and need for intensive care. If the use of biomarkers succeeds in preventing just one hospitalization, this could be a highly cost-effective strategy.

Study limitations

In our center, high-sensitivity troponin I has only been available since last year, so measurements at initial assessment are not available. However, plasma samples have been frozen in liquid nitrogen and a study with emerging biomarkers is ongoing. Due to the small population it was not possible to perform a multimarker score.

Conclusions

In our population, LVRR occurred in one third of DCM patients, especially in younger patients, with better renal function and smaller LVEDD. We can postulate that patients with long-standing disease and larger LV diameters may not recover LV function. CA125, BNP and hs-CRP were predictors of clinical severity but not of reverse remodeling. BNP correlated with parameters of systolic and diastolic dysfunction, while CA-125 correlated with measures of diastolic dysfunction.

Ethical disclosures

Protection of human and animal subjects. The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Confidentiality of data. The authors declare that they have followed the protocols of their work center on the publication of patient data.

Right to privacy and informed consent. The authors have obtained the written informed consent of the patients or subjects mentioned in the article. The corresponding author is in possession of this document.

Conflicts of interest

The authors have no conflicts of interest to declare.

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4. Profile of biomarkers of extracellular matrix, inflammation and apoptosis in left ventricular reverse remodeling

Sandra Amorim; Inês Falcão-Pires; Marta Oliveira; João Rodrigues; Manuel Campelo; Brenda Moura; Filipe Macedo; Adelino F. Leite-Moreira, J Silva-Cardoso; M Júlia Maciel

Under revision

ABSTRACT

Background: Limited data exists regarding the usefulness of novel biomarkers to predict left ventricular (LV) reverse remodeling (LVRR) in dilated cardiomyopathy (DCM). We aim to evaluate the potential of emerging biomarkers in LVRR.

Methods: We included 50 DCM patients with reduced LV ejection fraction (LVEF< 40%, 59±10 years, 56% males). We assessed LVRR (an increase>10 U of LVEF) and measured levels of markers of extracellular matrix (MMP-3, TIMP-2, ST2, Galectin-3) and HF (BNP, GDF-15, sTNF RI, CA 125), at baseline and 41.2±23.4 months after.

Results: LVRR occurred in 40% of patients within 17.6±15.6 months after pharmacological (n=17) and resynchronization therapy (n=3). Patients with pharmacological LVRR had younger age (60.6±8.8 vs 54.7±10.8, p=0.04), smaller LVDDi (mm/m2) (35.2±4.1 vs 32.3±4.8, p=0.03) and lower baseline galectin-3 (5.61±2.98 vs 8.68±4.35ng/ml, p=0.03). There was a significant decrease in BNP; but most biomarkers, particularly GDF-15 and MMP-3 (1140.01±928.45 vs 808.35±416.66 pg/ml;p=0.07; 10.1±6.0 vs 6.1±3.0 ng/ml;p<0.01), continued to rise, despite medical therapy, even in patients with LVRR.

BNP correlated directly with LVDDi (r=0.49), LVDV (r=0.51), PSAP (r=0.43), E/e´ (r=0,31); correlated inversely with LVEF (r=-0.50) and e´velocity (r=-0.32) (p<0.05). CA 125 correlated with LAVi (r=0,46), E/A ratio (r=0,60) and PASP (r=0,49) (p<0.05). GDF-15 correlated with E/e' (r=0.32, p=0.01); TIMP-2 correlated with LVDVi (r=0.31, p=0.01), LAVi (r=0.28, p=0.02) and sphericity index (r=-0.29, p=0.02).

Conclusions: Lower values of galectin-3 were associated with LVRR and correlations were found between biomarkers and echocardiographic parameters of LV remodeling, supporting the multidirectional pathway of remodeling in HF that involves cytokines, ECM activation and apoptosis. Despite LV systolic recovery, there is persistent diastolic disfunction, matrix fibrosis activation, apoptosis and inflammatory activity.

Acronyms and abbreviations: BSA: body surface area; DCM: dilated cardiomyopathy; LAVi: left atrial volume/BSA; LBBB: left bundle branch block; LVEF: left ventricular ejection fraction; LVDD: left ventricular end-diastolic diameter; LVDDi: left ventricular end-diastolic diameter/BSA; LVDV: left ventricular end-diastolic volume; LVDV: left ventricular end-diastolic volume/BSA; LVRR: left ventricular reverse remodelling; PASP: pulmonary artery systolic pressure; MR: mitral regurgitation

INTRODUCTION

Dilated cardiomyopathy (DCM) represents a common cardiomyopathy leading to heart failure (HF) and has an estimated prevalence of 1:2500 (1). In DCM, myocardial remodeling is characterized by ventricles enlargement, wall thinning, fibrosis as well as hypertrophy, slippage and loss of cardiomyocytes with subsequent systolic dysfunction (2). Treatment of DCM is essentially the treatment of chronic heart failure (HF), i.e., optimal pharmacological and/or resynchronization therapy, aiming for left ventricular (LV) reverse remodeling (LVRR). Indeed, LVRR, defined as a reduction in LV size with an improvement in systolic function, is a marker of a favourable prognosis in individuals with recent-onset DCM (3).

Currently, limited data exists regarding the utility of biomarkers to predict LVRR in DCM. So far, some studies have described potential biomarkers of reverse remodeling in several clinical contexts, but none were able to monitor LVRR progression or prognosis (4-7). Biomarkers of matrix remodeling and inflammation have emerged as potential preclinical indicators to identify individuals at risk of developing clinical HF (5, 8-10). Because LV remodeling seems to involve different pathophysiologic pathways, a multimarker panel meeting these requirements should help to refine risk stratification.

High-risk patients prone to LV remodeling could thus receive more aggressive therapy and more frequent follow-up. Recent evidence supported the utility of biomarkers in adjusting anti-remodeling treatments for specific groups of patients (11). Galectin-3 is a member of the galectin family that binds b-galactosides. In the failing heart, it is produced by macrophages and cardiac fibroblasts, an increase ofGalectin-3 promotes cardiac fibroblast proliferation, collagen deposition and ventricular dysfunction (10). Overexpression of matrix metalloproteinases (MMP)s may play an important role in ventricular remodelling, MMP-3 is important because of its ability to degrade a wide range of ECM components and to activate latent MMPs (12, 13). Tissue inhibitor of matrix metalloproteinases (TIMP)s have growth-promoting properties, stimulate fibroblasts differentiation and displays endogenous independent properties, promoting angiogenesis, regulating apoptosis, and amplifying inflammation (14, 15). ST2 is a member of the interleukin 1 receptor family and exists in two forms, a transmembrane receptor (ST2L) and a soluble decoy receptor (ST2). The ligand of ST2L is interleukin 33, which is involved in reducing fibrosis and hypertrophy in mechanically strained tissues. Excess soluble ST2 leads to cardiac fibrosis and ventricular dysfunction (16). Growth-differentiation factor 15 (GDF-15) is a distant member of the transforming growth factor-β cytokine superfamily and appears to be involved in the regulation of cell differentiation and tissue repair with possible anti-apoptotic and antihypertrophic effects and closely linked with tissue remodelling (17). Soluble TNF receptor TNFR1 has proapoptotic effects—facilitated cardiac remodeling and apoptosis in cardiomyocytes, while stimulation of TNFR2 has an antiapoptotic effect and their blood levels are elevated in patients with severe HF (18).

The aim of our study was to establish novel biomarkers of LVRR in the context of DCM. In this work, we evaluated the profile of emerging biomarkers in predicting LVRR, after optimal pharmacological or resynchronization therapy, in a cohort of ambulatory DCM patients.

We measured circulatory molecules related to biological pathways involved in HF: i) Galectin-3, MMP-3, TIMP-2, protein ST2 (ECM remodeling); ii) GDF- 15 (apoptosis), ii) sTNF-R1 and carbohydrate antigen 125 (inflammation); iv) BNP (myocardial overload).

METHODS

We included consecutive adult idiopathic DCM patients followed in a HF outpatient clinic, recruited from January 2011 to December 2014 in a tertiary care centre, with a diagnosis of less than 2 - 3 years duration. Patients must have had two initial values of LVEF lower than 40%, more than one year apart, to exclude reversible causes, like myocarditis. All patients were in sinus rhythm and patients with history of uncontrolled atrial and ventricular arrhythmias were excluded. December 2015 was considered the final follow-up date. We excluded DCM patients with secondary aetiologies, such as: 1) history of myocardial infarction or angina, with ischemia or significant coronary disease assessed by coronary angiography; 2) history of moderate to severe hypertension; 3) primary moderate-severe mitral or aortic valvular disease; 4) heavy alcohol use (>100 g/ day); 5) chemotherapy-induced and peripartum cardiomyopathy; 6) acute HF with positive biopsy for myocarditis or with positive serology for acute phase of bacterial or viral infection.

At baseline, patients underwent clinical assessment, EKG, transthoracic echocardiogram and blood collection for analytic measurements. Patients were managed according to current clinical practice guidelines (19) and clinicians adjusted the recommended target doses for all therapies. During the follow

up, periodic clinical evaluation, laboratory measurements and echocardiogram on a 3 to 6 months' basis were performed. All patients gave written informed consent. This study was performed in accordance with the recommendations set by the Declaration of Helsinki (20) and approved by the local Ethics Committee.

Definition of LVRR

LVRR was defined as an absolute increase of at least 10 units of LVEF in two subsequent echocardiograms, more than six months apart, concomitant with a decrease in diastolic left ventricular diastolic diameter (LVDD), LV systolic and diastolic volumes and LV mass, without worsening of mitral regurgitation (MR), if present.

Transthoracic echocardiography protocol

Transthoracic echocardiography was performed at baseline and during follow-up procedures using a commercially available echocardiographic system (General Electric Vivid 7.0) with a 2.5-MHz transducer. The chamber quantification parameters were measured according to the professional standards defined by the American Society of Echocardiography and the European Association of Echocardiography (21); LVEF (%) was calculated by Simpson's biplane method; degree of mitral and tricuspid regurgitation by Doppler, scored on a scale from 0 to 4; pulmonary artery systolic pressure (PASP) was calculated by tricuspid velocities. LV mass was calculated using the formula proposed by Devereux et al (22). LV sphericity index was calculated as the ratio of dimensions of long axis view and minor axis view. The early diastolic (E) and atrial (A) wave velocities, the E/A ratio, and the E-wave deceleration time were measured using pulsed wave Doppler recording from the apical four-chamber view. Spectral pulsed-wave Doppler–derived early diastolic velocity (e´) was obtained from the septal mitral annulus, and the E/e' ratio was calculated to obtain an estimate of LV filling pressure. The myocardial performance or Tei index was retrieved from the mitral flow pattern and calculated by the formula = (IVCT + IVRT)/ET, were IVCT is the isovolumic contraction time, IVRT corresponds to the isovolumic relaxation time and ET to ejection time. LV Tei index was measured at the septal and lateral sites of the mitral annulus and RV Tei index was determined as the difference in duration between tricuspid regurgitation and pulmonary ejection divided by pulmonary ejection duration. All data were digitally stored, and off-line double-blinded data analysis was performed by two echocardiography specialists. To assess the variability in interpretation, all echocardiograms were analysed independently by two echocardiography specialists, blinded to the study. The reproducibility of the measurements was calculated on the basis of standard error of the estimate; both interobserver and intraobserver variations were ≤5% for follow-up LVEF and LV volumes.

Biomarker´s assessment

All biomarkers were measured from plasma obtained at the time of study entry and at the end of follow-up. At baseline and follow-up venous blood samples were collected to ethylenediamine-tetra-acetic acid-containing tubes. After a 30 min-rest, blood samples were centrifuged at 5000 rpm for 15 min at 4°C, and plasma was collected and frozen at -80°C until analysis. BNP was measured by chemiluminescent immunoassay (Abbott). CA 125 was determined by chemiluminescence (Abbott). Plasma Soluble ST2 was quantified using a human IL-1 R4/ST2 enzyme-linked immunosorbent assay (R&D Systems, Abingdon, United Kingdom). Plasma MMP-3, TIMP-2, Galectin-3, GDF-15 and sTNF-R1 were also quantified by human enzyme linked immunosorbent assays (R&D Systems, Abingdon, United Kingdom) according to the manufacture instructions. Score ST2-R2, defined by Lupon et al (2), was not calculated due to differences between our immunoassay and the assay that they used in their study.

Statistical analysis

All values are reported as mean ± SD, median [interquartile range] or as percentages according to type of data. Differences between categorical data were assessed using X2 test. Groups were compared by two-way repeated measures ANOVA for baseline and follow-up analysis. Sequential comparisons within the same individual were assessed by a paired Student t test, Kolmogorov-Smirnov or Mann-Witney test after confirming normality and homogeneity of variance with Shapiro-Wilk and F tests, respectively. Bonferroni-adjusted t tests were used subsequent for multiple comparisons after repeated measure ANOVA, two-tailed P<0.05 was considered to indicate statistical significance. The correlation between plasma markers analysed and echocardiography parameters was analysed using the Pearson´s correlation test. Non-normally distributed continuous variables were log-transformed. Statistical analysis was performed with SPSS 23.0 statistical package (SPSS Inc., Chicago, IL, USA).

RESULTS

Population characteristics

We included 50 consecutive patients with DCM, 56% males, aged 59±10 years-old, mostly in NYHA class II (62%), followed for 39±22 months. On EKG, 66% patients had LBBB, all were in sinus rhythm. Mean left ventricular EF was 25.4±9.8%, LVDD was 62.4±7.4mm, LVDDi was 34.2±4.5mm/m2 and 34% had MR grade > II/IV. At baseline, 76% of patients were already treated with angiotensin-converting enzyme inhibitors (ACEI)/ angiotensin II receptor blockers (ARB), and 62% with β-blockers and 14% with mineralocorticoid receptor antagonists. At the end of the follow-up, 94% were on medication with ACEI/ ARB, 98% on β- blockers and 60% on mineralocorticoid receptor antagonists. Optimal recommended doses of ACEI/ARB were reached in 42% and optimal doses of β-blockers were reached in 48%. During follow-up 22% of patients were hospitalized for HF worsening, 4% died (n=2) and 40% received an implantable cardiac defibrillator (ICD). At the end of the follow-up, 15 patients (30%) had LBBB and a LVEF ≤35%, but only 8% received a cardiac resynchronization therapy plus ICD (CRT-D). The remaining patients didn´t implant the CRT because of: NYHA class I, technical problems, refusal of the device, transient LVEF recovery, presence of LV diverticulum and old age. Predictors of left ventricular reverse remodeling (LVRR) LVRR, defined as an increase of LVEF more than 10 units, occurred in 20 patients (40%) within 17.6±15.6 months. In 17 patients, it occurred after pharmacological treatment and in 3 patients (with no pharmacological LVRR) it occurred after CRT-D implantation. The baseline LVEF of patients who recovered LV function after medical therapy was like those who did not recover (24.9±9.0% vs 26.5±11.2%, p=0.58). We found that patients who recovered LV function had: younger age (60.6±8.8 vs 54.7±10.8, p=0.04), smaller LVDDi (mm/m2) (35.2±4.1 vs 32.3±4.8, p=0.03). Patients that had pharmacological LVRR had lower basal levels of galectin-3 (5.61±2.98 vs 8.68±4.35 ng/ml, p=0.03). We did not find other baseline clinical and echocardiographic factors that predicted LVRR. Further details are described in Table 1.

Patients with LVRR had a final EF of 48.9 ± 7.9% (Δ LV EF of 22.4%), and had a significant decrease ($p < 0.05$) (see Figure 1) in: LVDD (53.5 ± 6.7 mm; Δ LVDD of ± 7.2 mm), LVDDi (28.3± 3.0 mm/m2), LVDV (145.5 ± 32.7 ml), LVSV (73.6 ± 25.2 ml), LVMi (122.3±32.2 g/m2); and an increase ($p < 0.05$) in sphericity index (1.57 ±0.18) and only 5.9 patients (n=1) had a final MR ≥ grade II/IV. Controversially, in patients with reverse remodelled DCM, measures of diastolic function as LAVi, e' velocity and E/e' ratio, LV Tei index and RV Tei index were not significantly different from baseline. See Table 2 for further details.

Biomarker levels

BNP decreased significantly at the end of follow-up in patients who recovered LVEF (81.3±189.4 vs 20.6±54.5 pg/ml, p<0.05) but also in patients who didn´t recovered LVEF (171.2±530.1 vs 81.3±182.2 pg/ml, p<0.01). Paired analysis of novel biomarkers was only considered in 35 patients: 2 patients died suddenly and didn´t collect the final sample, the other patients had technical problems in collecting

samples or didn´t collect at the time of echocardiogram. Figure 2 and Table 3. displays the differences in the levels of plasma biomarkers at the baseline and at the end of follow-up, in patients with and without LVRR.

Mean baseline levels of galentin-3 were 7.81 ±3.66 ng/ml. Patients that had pharmacological LVRR had lower baseline levels of galectin-3 (vide supra). Although galectin-3 values seem to decrease during follow-up, it was not statistically different in patients with or without LVRR (Figure 2). At the beginning of follow-up, levels of MMP-3 were 8.05 ±5.11 ng/ml and all patients with levels more than 8 ng/ml were male. We found that MMP-3 significantly increased during follow-up both in patients with and without LVRR (No LVRR: 6.52±3.42 ng/ml vs 10.58±7.31 ng/ml, p=0.01; LVRR: 5.48±2.28 ng/ml vs 9.45±4.06 ng/ml, p<0.01), Baseline TIMP-2 levels were 87.92 ± 31.01 ng/ml and was not associated with LVRR. There also no significant changes during follow-up both in patients with LVRR (Figure 2). Baseline levels of ST2 were 17.79±8.1 ng/ml, were not associated with LVRR (18.56±6.65 vs 18.40±7.03, p=0.25) and there weren´t no significant changes during follow-up. In the case of GDF-15 (mean 1048.10±692.08 pg/ml) we found a tendency for an increase only in patients who recovered LVEF (1140.01±928.45 pg/ml vs 808.35±416.66 pg/ml; p=0.07). sTNF RI at baseline was 1105.86±414.87 pg/ml, we found a tendency for an increase over time, in the overall population (baseline: 10.73±3.32 vs final 11.62 ±5.00 ng/ml, p=0.057.

Correlations between biomarkers and echocardiographic parameters

However, some of the biomarkers showed important correlation with echocardiographic parameters of ventricular remodeling (Figures 3 and 4). Such is the case of BNP that correlated with BNP correlated directly with LVDD (r=0.49), LVDV (r=0.51), PASP (r=0.43), E/e´ (r=0,31); and was inversely correlated to LVEF (r=-0.50) and e´velocity (r=-0.32) (p<0.05). CA 125 positively correlated with LAVi (r=0,46), E/A ratio (r=0,60) and PSAP (r=0,49) (p<0.05). GDF-15 correlated with E/e' (r=0.32, p=0.01); GDF-15 positively correlated with E/e' ratio (r=0.32, p=0.01). TIMP-2 was weakly positively correlated with LVDVi (r=0.31, p=0.01), LAVi (r=0.28, p=0.02) and inversely with sphericity index (r=-0.29, p=0.02). MMP-3 had a weak positive correlation with LVEF (r=0.35, p=0.01). Controversially, ST2 had a weak negative correlation with LAVi (r=-0.26, 0=0.04) and a weak positive correlation with sphericity index (r=0.28, p=0.02). Moreover, certain plasma markers correlated with each other. Significant correlations were found between BNP and CA 125 (r= 0.64, p<0.01). CA 125 was positively correlated with GDF-15 (r=0.34, p=0.04) and with TIMP-2 (r=0.38, 0=0.02). sTNF RI was also correlated with TIMP-2 (r=0.34, p=0.01), with GDF-15 (0.50, p<0.01), Galectin-3 (r=0.31, p=0.01) and with MMP-2 (r=0.55, p=0.01). Lastly, GDF- 15 correlated with TIMP-2 (r=0.31, p=0.01).

Table 1. Baseline clinical, echocardiographic, biochemical characteristics and therapy of dilated cardiomyopathy patients separated according to the presence or absence of pharmacological left ventricular reverse remodeling (LVRR).

	No LVRR (n=33)	**LVRR (n=17)**	**p**
Age (years)	60.6±8.8	54.7±10.8	0.04
Male sex (%)	54.5	58.0	0.77
Mild Hypertension (%)	57.6	58.8	0.93
Heart rate (bpm)	77.7±14.9	73.6±11.3	0.98
Systolic blood pressure (mmHg)	124.5±18.0	123.4±21.7	0.85
QRS duration (ms)	140.6±27.7	136.1±33.8	0.13
LBBB (%)	69.7	58.8	0.44
NYHA class I (%)	27.3	17.6	0.45
NYHA class II (%)	57.6	70.6	0.37
NYHA class III -IV (%)	12.5	11.8	0.94
Biomarkers			
BNP (pg/ml) (median ± IQR)	171.1±530.1	81.3±198.4	0.50
CA 125 (U/ml)	32.7±76.6	47.8±152.1	0.65
sTNF RI/TNFRSF1A (pg/ml)	1140.98±314.00	958.47±341.04	0.11
TIMP-2 (ng/ml)	95.25±33.39	79.65±33.01	0.19
ST2 (ng/ml)	18.40±7.03	18.56±6.65	0.25
MMP-3 (ng/ml)	6.13±3.30	5.92±2.26	0.84
Galectin 3 (ng/ml)	8.68±4.35	5.61±2.98	0.03
GDF-15 (pg/ml)	1048.77±556.95	742.36±370.94	0.09
Echocardiogram			
LVEF (%)	24.9±9.0	26.5±11.2	0.58
LAVi (ml/m2)	70.3±26.3	67.4±25.0	0.47
LVDD (mm)	63.3±7.6	60.7±6.9	0.25
LVDDi (mm/m2)	35.2±4.1	32.3±4.8	0.03
LVMi (g/m2)	167.4±24.7	161.5±35.2	0.49
LVDVi (ml/m2)	111.6±30.0	106.4±27.3	0.57
LV Tei index	0.78±0.34	0.82±0.37	0.72
Mitral regurgitation ≥ grade II (%)	36.4	29.4	0.62
PASP (mmHg)	31.0±9.4	29.6±7.8	0.65
RV dimension (mm)	26.5±2.8	28.4±3.6	0.06
RV Tei index	0.46±0.16	0.52±0.32	0.42
RV S velocity (m/s)	0.13±0.02	0.12±0.02	0.56
E/e´	14.7±7.1	11.9±5.2	0.19
E´velocity (m/s)	0.07±0.03	0.07±0.01	0.86
Therapy Basal /Final (%)			
ACEI /ARB	81.8/93.9	64.7/94.1	0.18/0.98
Maximal dose ACEI/ARB	18.2/42.4	29.4/41.2	0.36/0.93
β- blockers	57.6/97.0	70.6/100	0.37/0.47
Maximal dose β-blockers	6.1/42.4	5.9/58.8	0.98/0.27
Aldosterone antagonist	12.1/63.6	17.6/52.9	0.59/0.46
Ivabradin	0.0/6.1	5.9/17.6	0.98/0.20
Diuretics	57.6/66.7	47.1/64.7	0.48/0.89
ICD	0.0/45.5	0.0/29.4	…/0.27
CRT-D	0.0/12.1	0.0/0.0	…./0.13

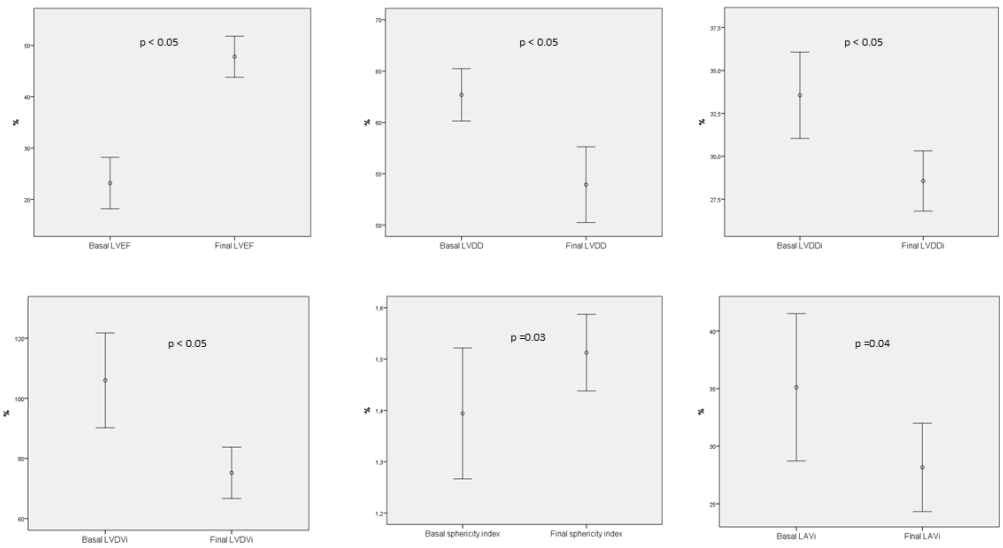
ICD/CRT: implantable cardiac defibrillator/ cardiac resynchronization therapy; LAVi: left atrium volume/body surface area; LBBB: left bundle branch block; LVDD: left ventricular end-diastolic diameter; LVDDi: left ventricular end-diastolic diameter/body surface area; LVDVi: Left ventricular diastolic volume/body surface area; LVEF: left ventricular ejection fraction; LVMi: left ventricular mass/body surface area NYHA: New York Heart Association; MR: mitral regurgitation; PASP: pulmonary artery systolic pressure;

Table 2. Comparison basal and final echocardiography parameters in patients with LVRR

n= 17	Basal	Final	p
LVEF (%)	26.5±11.2	48.9±7.9	**<0.01**
LVDD (mm)	60.7±6.9	53.5±6.7	**0.01**
LVDDi(mm/mm2)	32.3±4.8	28.3±3.0	**<0.01**
LVSD (mm)	54.4±7.9	41.5±4.6	**<0.01**
LVSVi(mm/mm2)	25.9±3.5	20.5±0.6	**0.02**
LVMi (g)	159.1±34.8	122.3±32.2	**0.01**
Sphericity index	1.44±0.22	1.57±0.18	**0.02**
LVDV (ml)	201.4±48.5	145.5±32.7	**<0.01**
LVSV (ml)	152.4±55.9	73.6±25.2	**<0.01**
LV Tei index	0.82±0.38	0.74±0.23	0.45
LAVi (ml/mm2)	35.6±14.4	28.8±3.8	0.27
E/e´ratio	11.6±4.6	10.47±4.2	0.49
e´velocity (cm/s)	6.7±1.5	7.7±2.2	0.14
RV Tei índex	0.56±0.35	0.39±0.17	0.13

LAVi: left atrium volume/body surface area; LVDD: left ventricular end-diastolic diameter; LVDDi: left ventricular end-diastolic diameter/body surface area; LVDVi: Left ventricular diastolic volume/body surface area; LVEF: left ventricular ejection fraction; LVMi: left ventricular mass/body surface area

Figure 1. Echocardiographic measures of left ventricular reverse remodeling (LVRR)



LAVi: left atrium volume/body surface area; LVDDi: left ventricular end-diastolic diameter/body surface area; LVDVi: Left ventricular diastolic volume/body surface area; LVEF: left ventricular ejection fraction.

Table 3. Baseline and follow-up biomarker data according to the presence or absence of left ventricular reverse remodeling (LVRR)

	No LVRR (n=19)			**LVRR (n=13+3) ***		
	Baseline	**Follow-up**	**p**	**Baseline**	**Follow-up**	**p**
BNP (pg/ml)	413.8±641.4	130.3±153.5	**0.04**	300.5±646.1	37.9±32.16	**0.01**
sTNF RI (pg/ml)	1097.6±283.2	1213.6±493.4	0.16	988.4±329.4	1097.4±518.0	0.22
TIMP-2 (ng/ml)	96.8±36.0	91.0±27.0	0.64	81.73±30.01	80.96±30.20	0.92
ST2 (ng/ml)	16.91±6.11	18.00±8.16	0.36	17.80±7.98	18.49±10.51	0.78
MMP-3 (ng/ml)	6.52±3.42	10.58±7.31	**0.01**	5.48±2.28	9.45±4.06	**<0.01**
Galectin-3 (ng/ml)	8.48±4.38	8.37±2.68	0.09	6.42±3.65	7.73±3.70	0.10
GDF-15 (pg/ml)	1041.6±570.4	1179.1±755.6	0.31	808.35±416.66	1140.01±928.45	**0.07**

* Included in this analysis 13 patients with LVRR after pharmacological treatment plus 3 patients with LVRR after CRT-D

Figure 2. Change in biomarkers from beginning to the end of follow-up in patients with and without left ventricular reverse remodelling (LVRR).

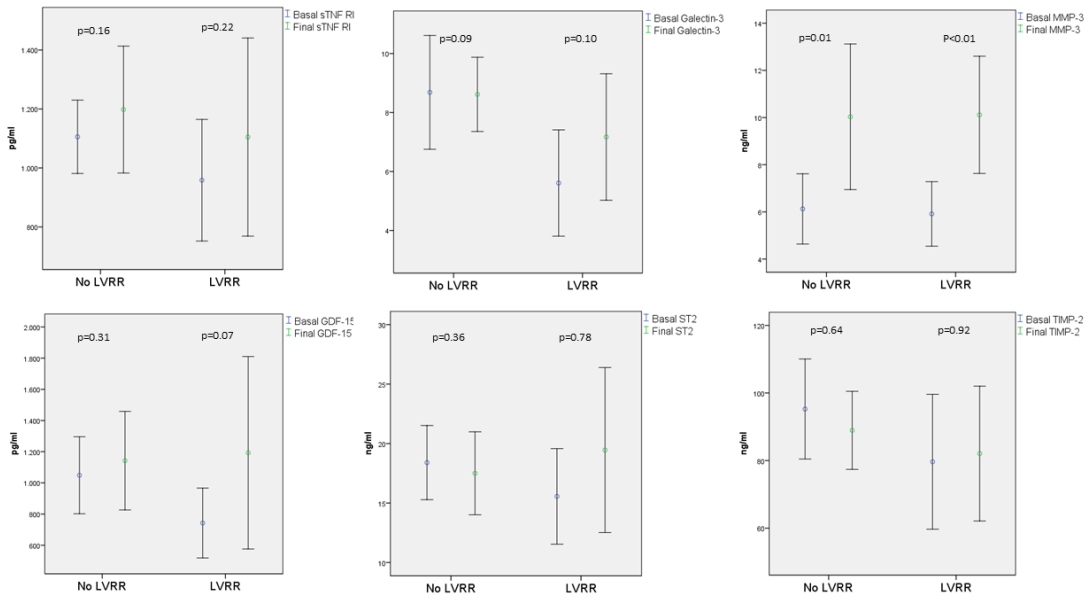


Figure 3. Correlations between BNP and CA 125 and echocardiographic parameters

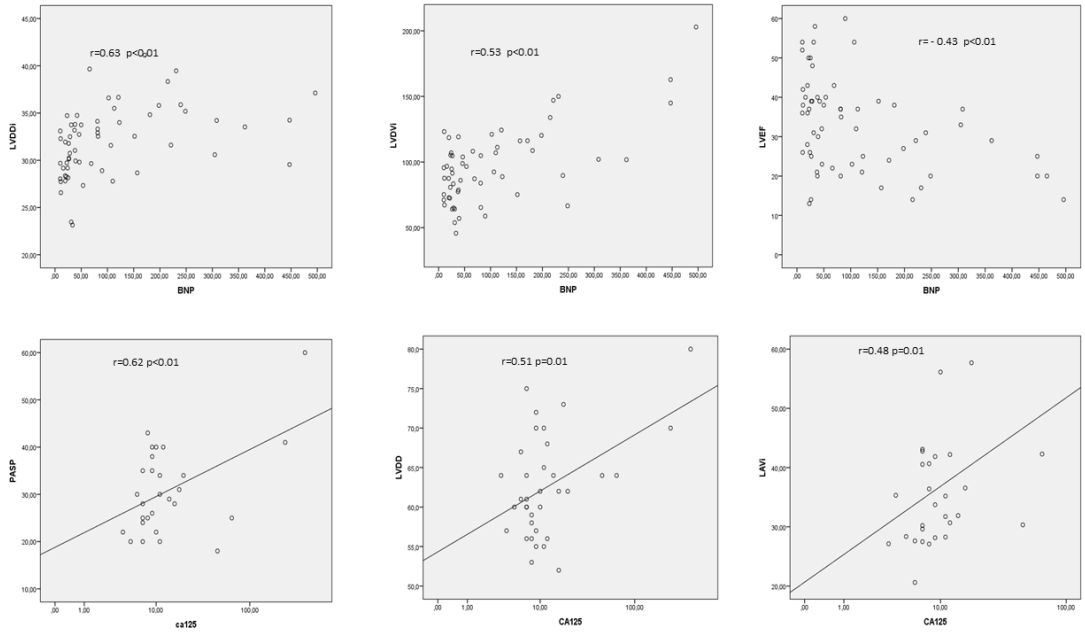
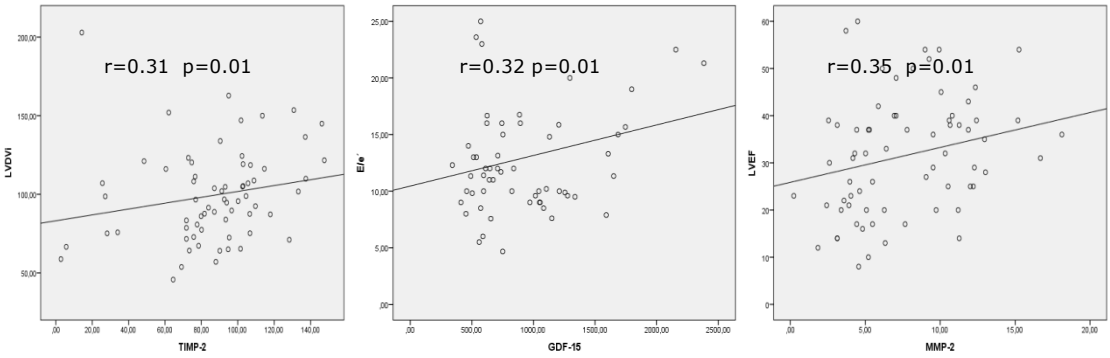


Figure 4. Correlations between GDF-15, TIMP-2, MMP-3 and echocardiographic parameters



DISCUSSION

After an initial injury to the myocardium, cardiac remodeling occurs, ultimately leading to left ventricular dysfunction and HF. Activation and proliferation of fibroblasts, which increase the synthesis of fibrillar collagen and activate the inflammatory response, play an important role (23). In our study, younger age, a smaller LVDDi and galectin-3 were the predictors of LVRR after pharmacotherapy, with lower levels being associated with a process of LVRR. Galectin-3 plays a role in this process, because it binds to ECM proteins and cell surface receptors (24). Lok et al. (10) found that patients whose LV volume decreased over time had significantly lower levels of galectin-3 at baseline. Experimental studies showed that inhibition of galectin-3 and aldosterone can reverse isoproterenol-induced left ventricular dysfunction in mice (25). This highlights the mechanistic link between the biological role of galectin-3 and cardiac fibrosis as well as its negative impact on cardiac structure and function. Controversially, plasma galectin-

3 did not change significantly there was no correlation between galectin-3 and echocardiographic measurements. This is consistent with studies in HF patients who failed to demonstrate any relationship between galectin-3 and LVEF (26). Weir et al (27) showed that plasma galectin-3 increased significantly over time and there was no correlation with LVEF or LV volumes on the baseline CMR scan, in patients with acute myocardial infarction (AMI) and LV dysfunction.

In our population, MMP-3 significantly raised during follow-up and those changes occurred both in patients with or without LVRR. In the literature, there are conflicting results between levels of MMPs. One large study in patients with AMI and MMP-3 measured at different time points from admission, showed that higher levels were associated with LV dysfunction, adverse LV remodeling and prognosis (28). Morishita et al. (29) studied 25 patients with successful reperfusion after an AMI and 15 normal controls, demonstrated that MMP-2 decreased to a minimum value within one week after the AMI, and then increased until 6 months after the AMI, which was ascribed to myocardial matrix degradation and a prolonged inflammation. In our study, TIMP-2 was correlated positively with LVDDi, LAVi and negatively with sphericity index, showing an association with pathological remodeling in DCM. In a recent study by Trucco et al. (30) with 42 patients that implanted CRT, TIMP-1 was a powerful predictor of long-term mortality. Another study showed that ΔTIMP-2 correlated with ΔLVESVi and with ΔLVEDVi and TIMP-2 levels, but started high and increased over time (31).

GDF-15 levels increased during follow-up, with a marginal difference in favour of patients who recovered LVEF; although higher levels of GDF-15 correlated with worse diastolic function (increased E/e'). In the Val-HeFT study, GDF-15 levels increased over the course of 12 months in patients randomized to the placebo arm (8). Although our lack of statistical difference was probably due to a small sample, our results can indicate a potential role of GDF-15 to track functional recovery during reverse remodeling. Similarly to other studies (32-35), BNP correlated positively with LV dimensions, LV volumes, LAVi and E/e´ratio, and negatively with LVEF; it is not surprising since the greatest secretion of B-type peptides is from the LV, in response to myocardial stretch. Serum CA125 is a tumor marker widely used in patients with ovarian cancer (36) and increased serum CA 125 values have also been shown in HF patients, caused by activation of the cytokine pathway (37). Serum levels of CA125 obtained in 286 HF patients correlated with CHF severity (NYHA class, e´ velocity, right atrial pressure) and with short-term prognosis (38). In our sample, CA 125 correlated mainly with measures of diastolic dysfunction. Our study was limited by the small number of patients, so there may be correlation between biomarkers and the occurrence of LVRR which was obscured. Comparing the baseline values of biomarkers in patients with and without LVRR, almost all biomarkers in the group who recovered LVEF had lower values, but only the levels of galectin-3 reached statistical significance. In the analysis of basal and final levels of biomarkers in our population, there was a significant decrease in BNP; but some biomarkers, particularly GDF-15 and MMP-3, continued to rise, despite HF therapy, even in patients with LVRR. We can speculate that the positive benefit on the myocyte cytoskeleton, in patients who recovered LVEF, goes beyond the aggravation of the other pathways or; despite ventricular reverse remodeling, there is persistent matrix fibrosis activation, apoptosis and inflammatory activity. Consistent to the last hypothesis are the results of The Penn Heart Failure Study, which included 1821 chronic HF patients (39). The HF-Recovered group was associated with a better event-free survival than HF-reduced EF and HF preserved EF groups; but this group still had abnormal BNP, uric acid, ST2, and soluble fms-like tyrosine kinase-1 and continued to experience HF hospitalizations, suggesting persistent HF risk (39). In another study, the recurrence of LV dysfunction, in HF recovered patients, was significantly correlated with the discontinuation of heart failure drugs (40). Also, despite the improvement in LVEF the risk of sudden cardiac death is continues (albeit at a lower level) (41). We therefore conclude that those findings provide a rationale to continue background medical or device therapy for HF-Recovered patients. Although LVRR refers to change in LV systolic function, LV size, volumes and mass, it is better to call it a "functional improvement" rather than true LV recovery. This is consistent with our data that diastolic function and myocardial performance indexes in patients with

reverse-remodelled DCM maintain impaired. Even though several biomarkers showed significant correlations with echocardiographic parameters and between each other, it was only a weak to moderate relationship. However, we still think that changes at the ECM can have an impact on myocardial function and structure recovery in DCM patients and our results corroborate the hypothesis of a multidirectional pathway of activation in HF that involves cytokines, ECM activation and apoptosis.

Study limitations

This was an analysis of a small, single-centre study. Our patient selection and definition of LVRR was strict and this may have conditioned the results. Measurement of biomarkers in two blood samples and non-uniform clinical follow-up time intervals might have limited the evaluation of changes of biomarkers over time. Lastly, it is not possible to know whether medications commonly used for HF can affect concentrations of the biomarkers.

CONCLUSIONS

Lower baseline values of galectin-3 were associated with reversal of LV remodeling, suggesting that galectin-3 may have a negative impact on cardiac function and lower levels of this marker may be a predictor of LVRR in DCM patients. Significant correlations were found between the levels of biomarkers and echocardiographic parameters of LV remodeling, supporting the multidirectional pathway of remodeling in HF.

Despite LV systolic recovery, there is persistent diastolic disfunction, matrix fibrosis activation, apoptosis and inflammatory activity.

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5. Left ventricular mechanical reverse remodeling not followed by electrical reverse remodeling

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Left Ventricular Mechanical Reverse Remodelling Not Followed by Electrical Reverse Remodelling: A Case Report

Sandra Amorim Raquel Garcia Teresa Pinho João Rodrigues Filipe Macedo José Silva-Cardoso M. Júlia Maciel

Cardiology Department, Centro Hospitalar São João, Faculty of Medicine, Universidade do Porto, Porto, Portugal

Established Facts

- In dilated cardiomyopathy patients, the left ventricular ejection fraction may improve or even normalize over time, and these patients would no longer be qualified for an implantable cardiac defibrillator based on the original criteria.
- The newly termed "heart failure with recovered ejection fraction" represents a distinct phenotype with biochemical properties and a natural history differing from those of the traditional heart failure population.

Novel Insights

- Besides recovery of the left ventricular ejection fraction to "normal values," in patients recovered from heart failure there still are abnormal circumferential and longitudinal left ventricular strain rates and left ventricular Tei index values, suggesting persistent subclinical left ventricular dysfunction with a maintained risk of arrhythmia.
- Left ventricular mechanical reverse remodelling is not always followed by electrical reverse remodelling, and advances in cardiac imaging may hold the promise of refining the determination of the risk of ventricular arrhythmias in dilated cardiomyopathy and aid in clinical decisions regarding device therapy beyond the ejection fraction.

Keywords
Reverse remodelling · Left ventricular ejection fraction · Implantable cardioverter-defibrillator

Abstract
Patients with severely depressed left ventricular ejection fractions (LVEFs) receive implantable cardioverter-defibrillators (ICDs) for the primary prevention of sudden death. However, in some patients, LVEFs may improve or even normalize over time, and these patients would no longer be qualified for ICD implantation based on the original criteria for which they have initially received an ICD. We report a patient with idiopathic dilated cardiomyopathy whose LVEF recovered to normal values after pharmacological therapy. Meanwhile, the patient had life-threatening ventricular fibrillation, aborted by the ICD. We reflect on the pathological features of left ventricular reverse remodelling and ventricular ar-

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E-Mail karger@karger.com
www.karger.com/crd

Sandra Maria Resende Amorim
Hospital de São João
Alameda Prof. Hernâni Monteiro
PT–4200-319 Porto (Portugal)
E-Mail sandra.maria.amorim@netcabo.pt

rhythmogenesis, where the myocardial substrate appears to play an important role. Also, after LVEF improvement in a patient with a cardiac device, there is still a debate on whether we should perform a battery replacement.

Introduction

The newly termed "heart failure (HF) with recovered ejection fraction" (HF-recovered) represents a distinct HF phenotype with biochemical properties and a natural history differing from those of the traditional HF population [1]. Factors that may influence left ventricular (LV) remodelling include ischaemia, infarction, fibrosis, inflammation, and others still to be defined. These elements which influence the composition of the myocardial substrate may be considered irreversible (e.g., fibrosis) or reversible (e.g., resolution of acute inflammation or reperfusion of ischaemic tissue) [2].

The underlying pathophysiological issue that needs to be addressed in patients with non-ischaemic dilated cardiomyopathy (DCM) is whether an improved LV ejection fraction (LVEF), either partial or complete, signifies substantial resolution of the cardiomyopathy substrate and its associated arrhythmia [3].

Case Report

A 65-year-old woman who was obese (BMI 42.4) with dyslipidaemia and hypertension had a long history of chest pain on exertion, and a previous stress test had shown left bundle branch block at the peak of the effort. Meanwhile, she had developed persistent left bundle branch block, with a QRS duration of 200 ms.

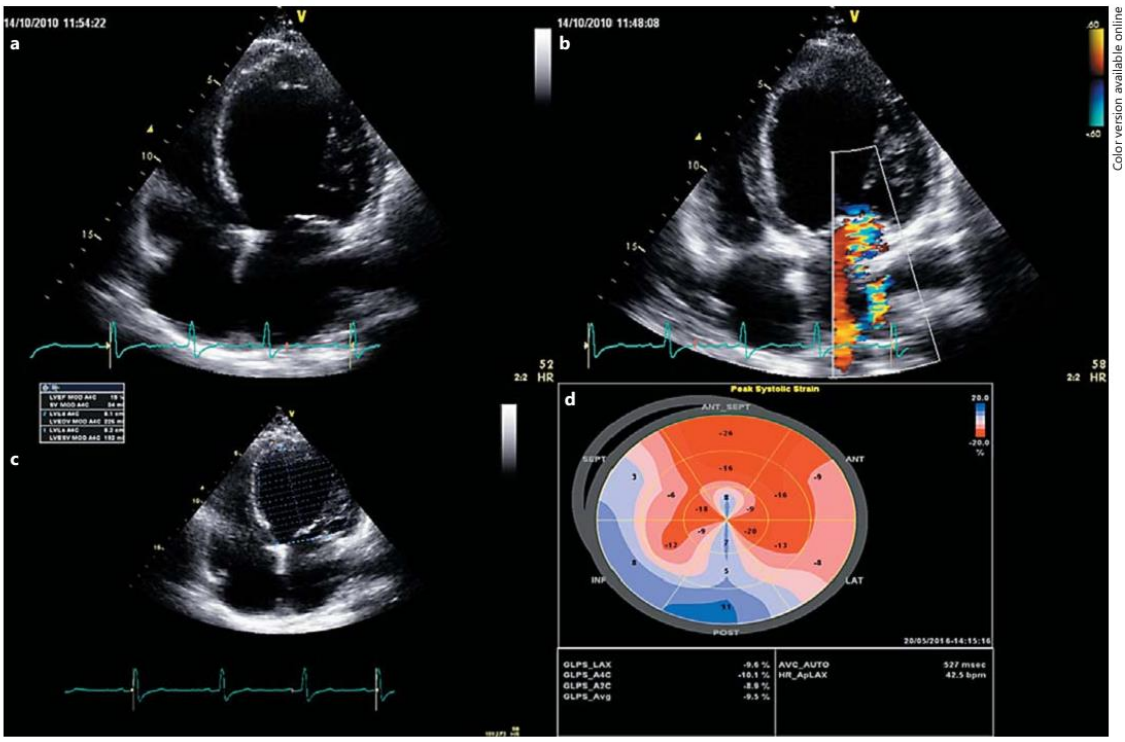

Fig. 1. a Transthoracic echocardiogram, apical 4-chamber view, showing severe dilatation of the left ventricle. **b** Mitral valve Doppler colour flow showing mitral regurgitation grade III/IV. **c** Left ventricular ejection fraction of 15%, calculated by biplane Simpson's method. **d** Bull's eye plot of peak systolic strain, with a left ventricular circumferential strain rate of –9.6% and a longitudinal strain rate of –9.5%.

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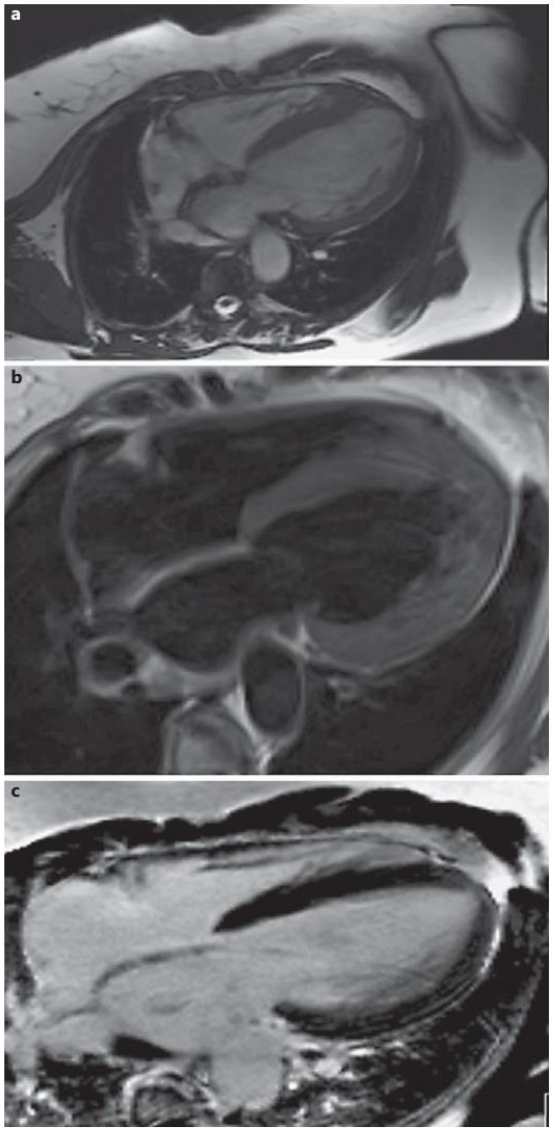

Fig. 2. a MR cine image showing dilatation of the left ventricle. **b** T2-weighted inversion recovery MR image with no signs of myocardial oedema. **c** Late gadolinium MR image showing no delayed enhancement.

The echocardiogram performed on October 2010 (Fig. 1) showed severe dilatation of the left ventricle (LV diastolic diameter 70 mm), light dilatation of the right ventricle, mitral regurgitation grade III/IV caused by annulus enlargement, an LVEF of 15% (biplane Simpson's method), an LV diastolic volume of 226 mL, an LV systolic volume of 192 mL, abnormal septal motion, and normal right ventricular function (S wave TDI 0.13 m/s). The values for the strain rate (speckle tracking) were as follows: an LV circumferential strain rate of –9.6% and a longitudinal strain rate of –9.5%. The LV Tei index was 1.33 and the specificity index was 1.09.

Coronarography showed normal coronary arteries and ventriculography revealed severe LV dysfunction. Cardiac MRI showed dilatation of the left ventricle (diastolic volume of 303 mL) with an EF of 38%, no signs of myocardial oedema, and no gadolinium delayed enhancement (Fig. 2).

Functionally, the patient was in NYHA class I–II and performed a cardiopulmonary stress test that showed a peak O_2 consumption of 16 mL/kg/min (91% of predicted for age and weight). HF therapy was optimized, reaching daily doses of 5 mg bisoprolol, 100 mg losartan, 40 mg furosemide, 25 mg spironolactone, and 40 mg simvastatin plus 100 mg ezetimibe.

The echocardiogram on May 2011 revealed a persistently depressed LVEF of 16%, an LV diastolic diameter of 65 mm, and mitral regurgitation grade III/IV. We decided to implant a cardiac resynchronization therapy-defibrillator (CRT-D), but we only placed the defibrillator lead in the right ventricle, because the patient was only mildly symptomatic.

On January 2013, the patient had an improvement in LVEF. The echocardiogram showed an LVEF of 32%, an LV diastolic diameter of 61 mm, and mitral regurgitation grade II/IV. The LVEF continued to improve, and, on January 2014, it was 42%.

On 3 November 2014, the patient had an appropriate shock caused by ventricular fibrillation (shown in Fig. 3). Echocardiography was repeated on 9 December 2014, revealing a normal LV function (LVEF 56%), an LV diastolic diameter of 56 mm, an LV diastolic volume of 155 mL, an LV systolic volume of 68 mL, and mitral regurgitation grade I/IV. However, the values for the strain rate and the Tei index were still impaired, i.e., an LV circumferential strain rate of –12.3%, a longitudinal strain rate of –10.9%, and an LV Tei index of 0.94 (Fig. 4). The last echocardiogram (January 2017) showed a persistently normal LVEF of 57% and an LV diastolic diameter of 54 mm. The patient maintained a left bundle branch block, with a QRS duration of 204 ms.

Discussion

Disease-related electrical remodelling is a fundamental mechanism underlying the proarrhythmic phenotype of HF. Reductions in both transient outward and delayed rectifier K^+ currents contribute to a prolongation of action potential duration (APD). A prolonged APD, in turn, promotes an increased influx of Ca^{2+} during excitation. Furthermore, prolongation of APD and abnormal handling of intracellular Ca^{2+} promote abnormal increases in focal activity and automaticity. In addition, hetero-

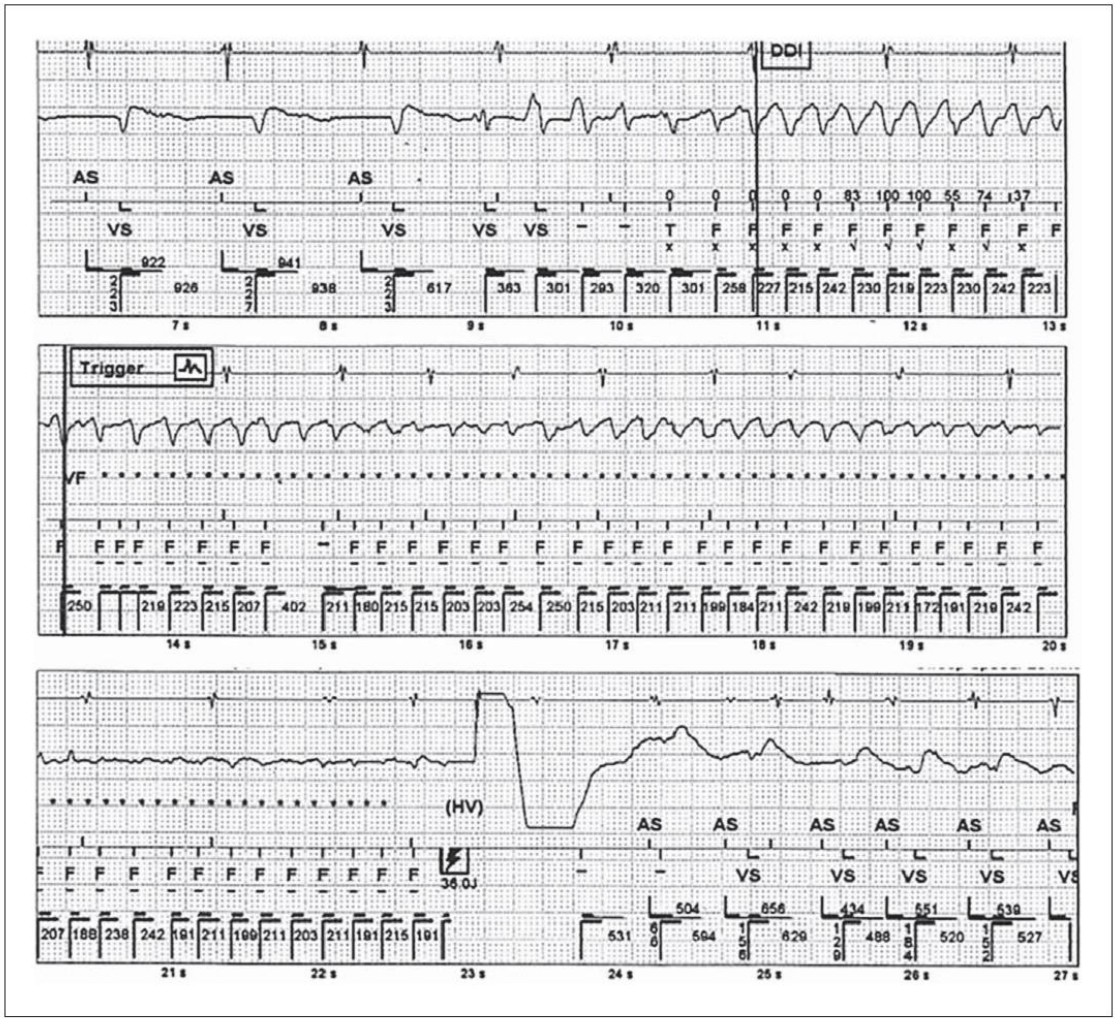

Fig. 3. Cardiac resynchronization therapy-defibrillator electrogram showing one episode of ventricular fibrillation, terminated with an appropriate shock of 36 J.

geneous APD prolongation within the ventricular wall amplifies dispersion of repolarization, an established mechanism contributing to re-entry. Finally, spatially different changes across the ventricular wall in HF alter the cellular coupling current. Together, these changes, along with the alteration in gap junctions and tissue alignment, lead to significant changes in electrical conductivity and sequence, which are important mechanisms underlying the increased propensity for ventricular arrhythmia and sudden cardiac death in HF [4]. The clinical efficacy of antiarrhythmic pharmacotherapy has proved disappointing in the majority of instances.

Our case report refers to a patient with idiopathic DCM with an LVEF recovery to normal values. We think, however, that we cannot totally exclude totally resolved myocarditis. Meanwhile, the patient had a life-threaten-

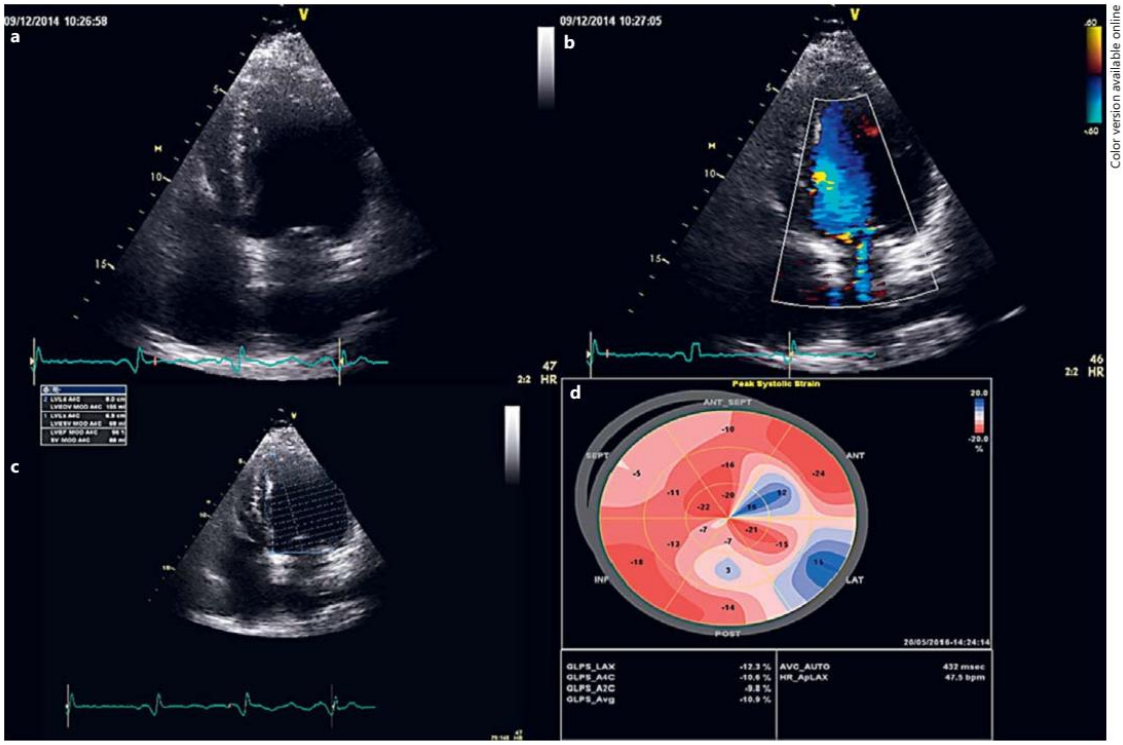

Fig. 4. a Transthoracic echocardiogram, apical 4-chamber view, showing a reduction of the left ventricular dimension. **b** Mitral valve Doppler colour flow showing mitral regurgitation grade I/IV. **c** Left ventricular ejection fraction of 56%, calculated by biplane Simpson's method. **d** Bull's eye plot of peak systolic strain, with a left ventricular circumferential strain rate of –12.3% and a longitudinal strain rate of –10.9%.

ing ventricular fibrillation, aborted by the cardiac device. Besides recovery of the LVEF to "normal values," we still measured abnormal LV strain rates and Tei index values; thus, we believe that the patient still had a subclinical LV dysfunction. In a study on patients with recovered LV function, subclinical LV dysfunction was observed at rest and during exercise [5]. Also, in a previously published study [6], in some patients whose LVEF had improved to normal values, measures of diastolic function, the myocardial performance index, and circumferential and longitudinal systolic strain rates were still impaired. We may therefore speculate that decreases in certain regional strain and strain rate parameters may be compensated by increases in other kinds of strain (e.g., radial strain) or LV torsion, playing a role in maintaining LVEFs in patients with HF-recovered.

Additionally, left bundle branch block may be related to the presence of fibrosis, which increases the arrhythmia risk. Contrary to our case, most of the studies showed that there is a significantly lower proportion of patients with left bundle branch block among those with LVEF improvement [7–9].

Future advances in refining risk factors for arrhythmias may include quantification of myocardial fibrosis and the extracellular matrix by late gadolinium enhancement cardiac MRI and characterization of the myocardial substrate by echocardiographic speckle tracking measures of global longitudinal strain and global circumferential strain [2]. Heterogeneity in the timing of regional mechanical forces in the radial axis or by longitudinal dispersion using strain imaging may be markers for ventricular arrhythmias. The LVEF is just one marker of

these disease processes. Electrolyte disturbances and stretch-induced arrhythmia may also contribute to arrhythmogenicity. Histological studies have shown increased fibrosis between cardiac muscle fibres and bundles that may enhance anisotropy and promote electrical uncoupling. This can provide the substrate for re-entry during ventricular fibrillation. Fibrosis might persist even in patients whose LVEF improves [10].

In theory, patients with a recovered LVEF do not have an indication for implantable cardioverter-defibrillator (ICD) or CRT-D implantation, and in several studies, there has been the question of timing of the implantation of these devices once LV dysfunction has been diagnosed. After LVEF improvement in a patient with a cardiac device, there is still a debate on whether we should perform a battery replacement; some authors do not support ongoing ICD therapy [11], whereas others take the viewpoint that continued ICD therapy is necessary even if the annual event rates are lower than in patients with a persistently low EF [10]. Although patients with preserved LVEF constitute >50% of the patients with HF, the data on the risk of sudden cardiac death among individuals with a preserved EF are sparse. In 1 study on 125 patients, 53 of the subjects (42%) received an ICD and 72 (58%) received a CRT-D for primary prevention; 21% had their LVEF normalized to ≥50%, 17% had their LVEF partially improved to 36–49%, and 63% had an LVEF that remained depressed at ≤35%. None of the individuals with a normalized LVEF received appropriate antitachycardia therapy regardless of whether they had an ICD or a CRT-D. Meanwhile, 20% of the patients with an LVEF at 36–49% and 14% of the patients with an LVEF at ≤35% received an appropriate ICD therapy [12].

A post hoc analysis of the DEFINITE study, which randomized patients with non-ischaemic cardiomyopathy to ICD versus medical therapy, found that patients with an improvement in EF (>5% increase) had significantly lower rates of all-cause mortality and arrhythmic death than those who had a stable EF or those with further reductions in EF; however, 17% of the patients with an improved EF still received appropriate ICD shocks during follow-up, compared to 27% with a stable EF and 33% with a worsened EF ($p = 0.45$) [7]. One study examined the incidence of appropriate ICD shocks among 91 patients with ICDs for the primary prevention of sudden death, and 27% of them had an improved EF (>10% absolute increase since original implantation and EF >35%) when they presented for elective replacement of their device [13]. During a 6-year follow-up since the first ICD implant, patients with and those without an improved EF had a similar incidence of appropriate ICD shocks and/or antitachycardia pacing. Chatterjee et al. [14] reported a meta-analysis focused on patients with LV reverse remodelling after CRT-D. Their major findings were that patients with LVEF recovery (defined as ≥35% or ≥45%) had significantly lower rates of ICD therapy for ventricular arrhythmias than patients without LVEF recovery ($p = 0.001$).

Most victims of sudden cardiac death have LVEFs which exceed the values of the current primary prevention guidelines for ICD implantation. Furthermore, Zecchin et al. [15] reported that appropriate ICD interventions for ventricular arrhythmias were documented in 11% of CRT-D recipients who were superresponders (LVEF >50%) after implantation. Therefore, they thought that it is not advisable to downgrade a CRT-D to a CRT pacing device at the time of generator exchange in a patient with recovery of the LVEF, because the risk of ventricular arrhythmias appears to remain. Collectively, these data show that approximately 20% of patients with LVEF improvement >35% are at risk of appropriate ICD shock/therapy, but the risk appears to be lower as the EF approaches the normal range [16].

Studies identifying risk factors for sudden cardiac death among patients with an improved LVEF are needed to help clinicians decide which patients are still at risk and would benefit from continuation of the ICD therapy. The data demonstrate that all-cause mortality rates are lower among patients with improving EFs [17]. Therefore, the life expectancy is lower, and thus prevention of sudden cardiac death among these patients may result in more quality-adjusted life years saved – favouring ICD implantation even if the annual event rates are lower. However, this must be balanced against the complication rates from ICD use.

Based on our case report, and together with follow-up studies of device therapy, we may speculate that LV mechanical reverse remodelling is not always followed by LV electrical reverse remodelling and that there still is an arrhythmia risk. Our recommendation is to continue background medical or device therapy for HF-recovered patients. Several advances in cardiac imaging may hold the promise of refining the determination of the risk of ventricular arrhythmias in DCM and aid in clinical decisions regarding device therapy beyond the LVEF.

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CHAPTER VI

DISCUSSION

1. DISCUSSION OF RESULTS

1.1 Prevalence, predictors and prognosis of reverse ventricular remodeling in idiopathic dilated cardiomyopathy

We studied the prevalence of LVRR in patients with Idiopathic DCM under optimized medical therapy, as well as it´s predicting factors and prognosis.

A total of 113 DCM patients were followed for 7.1±5.6 years. LVRR occurred in 34.5% of our patients after an average follow-up of 22.6 months.

On univariate analysis, the predictive factors for LVRR ($p<0.05$) were the presence of mild hypertension, atrial fibrillation, ECG criteria of left ventricular hypertrophy, shorter QRS duration, absence of left bundle branch block, higher haematocrit value, lower LV Diastolic Diameter index, higher peak oxygen uptake efficiency (VO2/log10[VE]), lower dVE/VCO2/VO2, treatment with ACEI)/ARB and the use of ACEI/ARB and β-blockers at maximal dosages. Multivariate regression analysis showed that the use of higher ACEI/ARB doses was independently associated with LVRR (OR: 0.32, 95% CI 0.11--0.92).

LVRR was associated with an improvement in NYHA functional class, a decrease in BNP values and a much better prognosis as opposed to what observed in patients in whom LVRR did not occur. In a meta-analysis, by Kramer et al., including 118 clinical trials (n=89,687 patients; median follow-up 17 months) survival was correlated with therapy-derived improvement on LVEF ($r=0.51$, $p=0.001$), LV Diastolic Volume ($r=0.44$, $p=0.002$), and LV Systolic Volume ($r=0.48$, $p=0.002$) (173).

The prevalence and the predictors of reverse remodeling in patients with DCM is not well established since the various studies previously published are not homogeneous in terms of the definition of reverse remodeling, of the populations studied, of the treatment applied and of the duration of the follow-up (172). These studies included heterogeneous populations with a variable ratio of patients presenting dilated cardiomyopathy of recent onset or secondary to reversible causes such as viral myocarditis, or toxic insult (54, 56). These are patients a higher potential for LVRR, either because of resolved activity of underlying disease (as in the case of acute myocarditis) or because of the favorable effects of neuro-hormonal blockade. This was why we specifically studied a homogeneous population of patients with IDCM under optimized medical therapy and excluded those in which IDCM was of a recent onset (172).

LVEF recovery and reverse remodeling were associated with the use of ACEI/ARB and β-blockers at maximal tolerated dosages. Experimental evidence suggests that β-blockers can increase the production of sarcoplasmic reticulum calcium ATPase mRNA and α-myosin heavy chain mRNA and decrease the synthesis of β-myosin heavy chain mRNA (8); these changes translates in an improved contractility and in recovery of the failing cardiac myocytes.

Our results are consistent with the literature investigating the clinical variables associated with LVRR in DCM (174-176). Cicoira et al (177) in 98 patients with IDCM, showed that LVRR tended to occur in patients with a shorter duration of symptoms, a worse NYHA class and history of hypertension. In another study (178), LVRR was found in 89 (37%) of

242 patients with IDCM and the baseline predictors of LVRR were a higher systolic blood pressure and the absence of left bundle branch block. Binkley et al. (179) showed that patients experiencing LVEF recovery were younger and more frequently female, had a lower prevalence of diabetes and of ischemic heart disease and a higher prevalence of hypertension, and had higher systolic blood pressure, lower serum creatinine level and shorter QRS interval.

In DCM, LBBB may be either causative or secondary to the underlining disease. LBBB may in fact be the cause of non-ischemic cardiomyopathy and in selected patients its resolution through CRT has been associated with normalization of LV function. On the other hand, DCM is associated with ventricular rearrangements and conduction delays, ultimately leading to LBBB. In our population, mean QRS duration of patients who did not recover LV function was 130 ms and this finding is consistent with the recommendations for biventricular pacing.

There is not a definite agreement in the literature regarding cardiac MRI late gadolinium enhancement (LGE) as a predictor of LVRR. Kubanek M et al. observed that in patients with recent-onset DCM (some of them with myocarditis) low extent of LGE and high edema ratio on CMR imaging were the most powerful baseline predictors of LVRR (56). In a study of 97 patients with DCM, LVRR (increase in LVEF of 5%), neither baseline LVEF nor the presence of CMR-LGE (88 patients) predicted LVRR (180). In another sub-study of 66 DCM patients LGE was detected both in the patients who did not experience LVRR as well as in patients with late LVRR, although the extent of LGE enhancement was lower in patients who responded compared to the non-responders (181). In our study, we failed to demonstrate that the presence or the extent of LGE on cardiac MRI as a predictor of LVRR, but possibly this was due to the small sample of our population.

In summary, in IDCM LVRR occurs in approximately one third of the patients under maximally tolerated medical therapy. A set of variables can discriminate patients in whom LVRR is likely to occur from those in whom it is not and who may require CRT therapy or more aggressive strategies, like heart transplantation. Current guidelines suggest that ICD/CRT should only be implanted after reassessment of LVEF following titration to maximally tolerated doses of medical therapy. Since, as above described, we may be able to discriminate patients that are likely to recover LVEF from those who are not, we may hypothesize that those with a more severe status, like those with LBBB, low systolic blood pressure, or larger left ventricular diameters, should implant an ICD/CRT at an earlier occasion, since it is relatively unlikely that they recover LV function only under medical therapy.

1.2 Persistent subclinical myocardial systolic and diastolic dysfunction after left ventricular reverse remodeling in idiopathic dilated cardiomyopathy

LVEF is not the most precise indicator of remodeling, as other metrics, such as LV volumes and mass, relate more closely to prognosis and to the impact of therapy (170). The LVEF value depends closely and inversely on LVEDV. For the same stroke volume, LVEF will be greater if LVEDV is smaller and vice versa. Thus, LVEF is unable to predict the magnitude of stroke volume, which is the true indicator. However, LVEF give us a standardized care and

implement quality assurance, it is not a limitation, but a tool. In ongoing and planned HF trials, EF remains a fundamental criterion.

Evaluating LV function strain rate imaging has a theoretic advantage over Doppler tissue imaging because it is relatively immune to cardiac translational motion and tethering (182). Several authors showed that in patients with HF global strain is a powerful predictor of cardiac events with a better accuracy than LVEF (183-185). Circumferential and longitudinal speckle-tracking strain rate analysis can be useful to detect subclinical myocardial systolic and diastolic dysfunction.

In idiopathic DCM, echocardiographic myocardial deformational parameters were insufficiently studied. This is the case of regional myocardial function and strain rate analysis in patients with normalized EF after optimal pharmacologic therapy. In these patients, Okada M et al. demonstrated the persistence of subclinical LV dysfunction using strain rate analysis at rest and during exercise (186).

We performed a prospective study including 50 DCM patients in sinus rhythm. LVRR occurred in 34% of them within 17.6±15.6 months and was associated with a reduced rate of death or HF hospitalization (5.9 % vs 33.3; p=0.03). Patients with LVRR had a final EF of 48.9 ±7.9% (Δ LVEF of 22.4%) and there was a significant decrease ($p<0.05$) in LVDDi, LVSD, LVDV, LVSV and LV mass and an increase ($p<0.05$) in sphericity index. However, measures of diastolic function (LAVi, e´velocity and E/e´ratio), final LV and RV Tei indexes and SSRcirc and SSRlong values were not significantly different from baseline. Even in patients in whom LVEF recovered to reach values of LVEF> 50%, SSRcirc and SSRlong remained below normal.

These findings suggest that in patients with IDCM after the occurrence of LVRR associated with medical therapy, left ventricular function may remain abnormal, even when LVEF returned normal. This fact may be in favour of maintaining neuro-hormonal blockade even after LVEF recovery.

Myocardial imaging studies have demonstrated a significant decrease in LV myocardial longitudinal and circumferential strain, as well as in LV twist in HFrEF compared with HFpEF. In both in ischaemic HFrEF and DCM, besides the compromised global and regional changes in longitudinal deformation (more preserved longitudinal strain in the LV base compared to the LV apex), LV apical rotation and overall LV systolic twist are reduced, resulting in a proportional decrease in LVEF. In severe cases, apical rotation may be so impaired that the LV base is the primary determinant of overall LV twist. The LV untwist at the macroscopic level amplifies the elastic recoil occurring at the microscopic level due to the rapid uncoupling of actin myosin cross-bridges and restoration of titin molecules to their original shape (169). So, multidirectional myocardial analysis may be important for a better understanding of subclinical myocardial dysfunction in patients with HF. We didn´t perform radial strain or LV torsion due to software limitations; those parameters may be important for the comprehension of the mechanism of reverse remodeling in DCM patients.

Foreshortened views and the assumption that speckles can be tracked from frame to frame, despite their out of plane motion, may affect circumferential strain measurements from the short-axis view. These limitations can be overcome by three-dimensional echocardiography and cardiac MRI.

1.3 Biomarkers in dilated cardiomyopathy: predictors of pharmacological reverse remodeling and clinical severity

In IDCM, many biomarkers may be used to assess various pathogenic pathways, including neurohormonal activation, oxidative stress, interstitial matrix remodelling and myocyte stretch and injury. Biomarkers profile of patients with HF has been investigated to stratify prognosis. Additionally it has been used in an effort to identify patients who might respond best to therapeutic interventions whether it is drug or device therapies (47). We evaluated the value of biomarkers in the prediction of the occurrence of LVRR.

In our prospective study including 50 DCM patients BNP, 25-OH-vit D, CA 125, hs CRP, Lp (a) values could not predict the occurrence of LVRR.

Patients in NYHA class III-IV, with pulmonary congestion or ankle oedema had higher levels of CA 125, cystatin C, BNP and hsCRP ($p<0.05$). CA 125 was correlated with BNP levels, with hsCRP and uric acid ($p=0.01$). BNP correlated directly with LVDD, LV volumes, PSAP, E/e´ and was inversely correlated to LVEF and e´velocity ($p<0.05$). CA 125 positively correlated with LAVi, E/A ratio and PSAP ($p<0.05$). There was a marginal correlation between hsCRP and LVEF and LVDVi. We found that levels of 25-OH-Vitamin D were only correlated with PSAP and E/A ratio ($p<0.05$).

ANP and BNP are produced in response to myocardial stretch due to pressure or volume overload (49). Similarly to our study the ICON study showed that HF severity, as measured by NYHA functional class (187) correlated directly with increasing values of BNP (188). Also in alignment with our data other studies (189, 190) demonstrated that BNP and NT-proBNP values correlate positively with LV dimensions, LV volumes, and LV mass and are inversely correlated with LVEF; BNP has shown to have the strongest correlation with LV diastolic wall stress which is consistent with stretch-mediated BNP secretion (189, 190). Similarly to our study others also showed that BNP levels increase with diastolic dysfunction severity, and correlate with indexes of LV filling pressure as well as with indexes of compliance and myocardial relaxation (191, 192).

Serum CA 125 is a tumor marker widely used in patients with ovarian cancer (193). In HF it is still unclear biologic role of this substance, namely if it simply reflects an activation of the cytokine pathway or if it is indeed responsible for myocardial and/or skeletal muscle dysfunction. In our population, CA 125 was not a predictor of LVRR, but was a predictor of a more severe HF presentation manifested by more severe functional class and pulmonary and peripheral congestion. CA 125 correlated with measures of diastolic dysfunction: LA volume, E/A ratio and PSAP. It also correlated with BNP, hsCRP and uric acid values.

CRP is a plasma protein participating in the systemic response to inflammation, a very important pathophysiologic mechanism of HF progression. In our patients, hsCRP was associated with HF clinical severity and marginally correlated with markers of LV systolic dysfunction: LVEF and LVDVi. CRP was previously shown (194) to be positively associated with NYHA class and to a higher risk of HF-hospitalization and mortality. In HF ACEI and β-blocker therapy have been associated with lower levels of CRP (195) . Now, it remains unclear

if CRP is merely a marker of inflammation or if, on the contrary it is involved in the pathogenesis and the progression of HF.

Functional vitamin D receptors are present in cardiac cells and their activation affects gene expression, proliferation and contraction of cardiomyocytes. In this context, vitamin D might contribute to the development of cardiac hypertrophy and fibrosis (196). In our population, we found that 25-OH- Vitamin D was not a predictor of LVRR and only correlated with PSAP and E/A.

As a conclusion, some of the biomarkers may reflect HF severity rather than a marker of reverse remodelling or recoverability.

1.4 Extracellular matrix, inflammation and apoptosis biomarkers profile and left ventricular reverse remodeling

Limited data exists regarding the usefulness of novel biomarkers to predict LVRR in DCM. We evaluated the potential of emerging biomarkers to predict the occurrence of LVRR.

In a group of 50 IDCM patients we measured the levels of extracellular matrix markers (MMP-3, TIMP-2, ST2, Galectin-3), GDF-15 and sTNF RI at baseline and after 41.2±23.4 months of follow-up. Patients presenting LVRR during the follow-up had lower baseline galectin-3 (5.61±2.98 vs 8.68±4.35ng/ml, p=0.03). During follow-up, GDF-15 increased (marginally) in patients with LVRR and MMP-3 increased in the overall population. GDF-15 correlated with E/e', TIMP-2 correlated with LVDVi, LAVi and sphericity index.

Galectin-3 is likely to play a role in cardiac remodeling, because it has a unique chemical structure, which makes it able to bind with extracellular matrix proteins and cell surface receptors (197). In our study, a low Galectin-3 value was the only predictor of the occurrence of LVRR after medical therapy. Other studies demonstrated that galectin-3 has a negative impact on cardiac structure and function. This is in alignment with our study where low baseline galectin-3 values were associated with the occurrence of LV reverse remodeling during follow-up. We can speculate that galectin-3 blockade might favourably affect remodelling and can be a future target for treatment.

In our population, MMP-3 significantly raised during follow-up both in patients with or without LVRR. We could not find studies on the behaviour of MMP-3 and TIMP-2 in LVRR in IDCM patients. There are also conflicting results regarding MMP-2 and MMP-3 levels in HF patients.

In our study, TIMP-2 was positively correlated with LV diastolic volume, LV diastolic volume/BSA and inversely with sphericity index. Weir et al (202) analysed TIMP-1, -2, and -4 behaviours in patients with LV remodelling and systolic dysfunction after an AMI. TIMP-2 concentration did not correlate with any LV functional parameter or with infarct volume index at baseline or at 24 weeks. In this study ΔTIMP-2 did correlate with ΔLVESVI and with ΔLVEDVI though not with ΔLVEF or with change in infarct volume index. They observed that TIMP-2 levels were high at baseline and increased over time.

GDF-15 is a member of the transforming growth factor-β cytokine superfamily and appears to be involved in the regulation of cell differentiation and tissue repair with possible anti-apoptotic and anti-hypertrophic effects and is closely linked with tissue remodelling (205).

In the Val-HeFT study, GDF-15 levels increased over the course of 12 months in patients randomized to the placebo arm (203). In our study GDF-15 levels increased during follow-up, with non-significant lower values in patients with LVRR. We may hypothesise that this lack of statistical significance difference was due to a small sample size and that GDF-15 may in fact be able to track LVRR. In our patients, higher levels of GDF-15 correlated with worse diastolic function indexes (increased E/e').

In summary, in our study most of the baseline novel biomarkers values were lower in patients in whom LVRR occurred during follow-up versus those in whom it did not, but statistical significance was only reached in the case of galectin-3. Our study was limited by a small sample size, which may have obscured a possible correlation between other biomarkers' values and the occurrence of LVRR.

In our population, when comparing biomarkers basal and final levels, GDF-15 and MMP-3, continued to rise, despite HF therapy, even in patients with LVRR. We can speculate that despite LVRR, there is persistent matrix fibrosis activation, apoptosis and inflammatory activity. Consistent to this hypothesis are the results of The Penn Heart Failure Study, which included 1821 chronic HF patients (79); in the group presenting LVRR an improvement in prognosis was observed, nevertheless this group still had abnormal BNP, uric acid, ST2, and soluble fms-like tyrosine kinase-1 and continued to experience HF-hospitalizations, suggesting persistent HF risk despite LVRR (79).

We found significant correlations between CA 125 and GDF-15 and with TIMP-2; STNF RI, TIMP-2 and GDF-15 were also correlated with each other. This is consistent with a multidirectional pathway in HF pathogenesis involving cytokines, ECM activation and apoptosis.

1.5 Persistence of Left Ventricular electrical remodeling despite Left Ventricular mechanical reverse remodeling

According to current international HF guidelines an ICD implantation is indicated in patients with HF and severely depressed LVEF for the primary prevention of sudden cardiac death. In some patients, LVEF may improve or even normalize over time and these patients would no longer be qualified for ICD implantation.

Our case report describes a patient with idiopathic DCM in whom LVEF recovery to a normal value occurred. Despite this the patient had a life-threatening ventricular fibrillation, aborted by the ICD he had implanted when he still had severely compromised LVEF. Despite the recovery of LVEF to a normal value abnormal LV strain rate and Tei index values were still present, indicating the persistence of subclinical LV dysfunction.

Future advances to refine arrhythmias risk prediction may include quantification of myocardial fibrosis by late gadolinium enhancement on cardiac MRI imaging and myocardial substrate characterization by echocardiographic speckle tracking measures of global longitudinal strain and global circumferential strain (206). Heterogeneity in timing of regional mechanical forces in the radial axis or longitudinal dispersion using strain imaging may be markers of risk for ventricular arrhythmias. While current prognostic and therapeutic recommendations are largely dependent on symptoms and on LVEF, come studies highlighted LGE as a crucial parameter for risk stratification and, possibly, for device implantation. Thus,

several advances in cardiac imaging beyond LVEF may hold promise in refining risk quantification for ventricular arrhythmias in DCM and aid in deciding ICD implantation.

Multiple studies have demonstrated an association between midwall fibrosis on LGE-CMR imaging and SCD events in patients with DCM (207) In the study of Halliday B et al (208) midwall LGE identifies a group of patients with dilated cardiomyopathy and an LVEF ≥40% at increased risk of SCD who may benefit from ICD implantation.

In a HF patient with an ICD, if LVEF improvement occurs and when the battery runs out there is an ongoing debate if we should replace the battery. Some authors suggest that we should not (209), whereas others think we should (210) . Chatterjee et al. (211) in a meta-analysis including patients experiencing LV reverse remodelling after the implantation of a CRT-D found that patients with LVEF recovery (defined as LVEF ≥35% or ≥45%) had significantly lower rates of ICD therapy for ventricular arrhythmias as compared with patients without LVEF recovery (p= 0.001). Nevertheless, Zecchin et al. reported that appropriate ICD interventions for ventricular arrhythmias occurred in 11% of CRT-D recipients who were super-responders (LVEF >50%) to device therapy (212). This is in favour of maintaining a CRT-D strategy at the time of generator change in a patient experiencing LVEF. Collectively, these data show that approximately 20% of patients with LVEF improvement above 35% remain at risk of appropriate ICD therapy, but the risk appears to be lower as the EF approaches normal range (213). As all-cause mortality is lower in patients with improving LVEF (173), life expectancy is longer and so the prevention of sudden cardiac death may result in more quality life-years saved, favouring ICD implantation. However, this must be balanced against the complication rates of ICDs.

In summary, LV mechanical reverse remodeling is not always associated with LV electrical reverse remodeling and an arrhythmic risk may persist.

2. MYOCARDIAL RECOVERY AND MYOCARDIAL REMISSION

HF patients undergoing LVRR, either spontaneously or after pharmacological or device therapies, may follow one of two potential avenues: 1) freedom from future HF events or 2) recurrence of HF events.

Taking in account these two different paths after the occurrence of LV reverse remodeling, Mann et al. (22) suggested that the term "myocardial recovery" should be applied in the case of the normalization of molecular, cellular and LV geometric changes allowing the heart to maintain preserved LV structure/function in the face of normal and/or perturbed hemodynamic loading conditions.

Accumulating evidence shows that the molecular changes associated with HF, in particular at the transcriptome, metabolome, and ECM levels, persist in the reverse-remodelled myocardium despite apparent normalization of macrolevel properties (74).

In 2005, a transcriptome analysis of 199 human myocardial samples from failing, nonfailing, and mechanically unloaded hearts showed significant HF-related changes in the expression of >3,000 genes, however, mechanical unloading did not induce a generalized 'transcriptional recovery', with only 5% of those genes showing normalization of expression levels after LVAD support. In addition, a new subset of genes was dysregulated in the reverse-remodelled, LVAD-supported heart compared with normal myocardium (214).

LV structural and functional improvements were accompanied by a new set of expressed genes related to the ECM, cytoskeleton, sarcomere, and excitation–contraction coupling. As more of the HF-related gene programme returned to normal values, the hearts had a greater capacity to withstand a second haemodynamic stress. Patients previously 'recovered' can latter suffer HF recurrence due to re-emergence of HF-associated transcriptional abnormalities (74).

Matricellular proteins (osteopontin, SPARC thrombospondins, tenascin, and periostin) interact with cell-surface receptors, growth factors, and other ECM proteins, and function as a link between ECM proteins and cardiomyocytes to modulate cell behaviour. Matricellular proteins levels increase in response to stress, independently of ECM and fibrillar content changes. Abnormalities in nonfibrillar ECM components persist after LVAD-induced reverse remodeling (215).

Taking the above into consideration, the term "myocardial remission" should be used to refer to the recrudescence of the molecular, cellular, and LV geometric changes that are insufficient to prevent the recurrence of HF in the face of normal and/or perturbed hemodynamic loading conditions. Although myocardial remission may be associated with stabilization of HF clinical course as well as reversal of many aspects of the HF phenotype, it is not associated with freedom from future cardiac events (22).

3. QUESTIONS UNANSWERED AND FUTURE RESEARCH

Normal LV function is conditioned by LV double helicoidal architecture, which is determined by the non-contractile LV myocardial components. In concentric LV hypertrophy, the double helicoidal architecture is preserved resulting in normal or near normal LV twist and LVEF, whereas in eccentric LV hypertrophy, the double helicoidal orientation is disrupted resulting in decreased LV twist and LVEF. In HFrEF, therapeutic interventions that retard or inhibit ECM remodeling have proved effective, whereas those that increase myocardial contractility with no effect at ECM level are ineffective (169). It is time to start thinking of a new, pathophysiologically driven classification, which will take into consideration several parameters of LV morphology and function.

Disease progression may also occur even in the absence of progressive cardiac remodeling. Although the biological motifs that separate reversible (elastic) from irreversible (plastic) changes in the heart are not known, it is likely that the progressive loss of cardiac myocytes, irreversible changes at the DNA level, and the progressive erosion of the native 3-dimensional organization of the ECM surrounding the cardiac myocytes will be critical determinants that distinguish between myocardial remission and myocardial recovery (8, 216).

There are two ongoing randomized studies testing withdrawal of medical therapy in patients who recovered from DCM. They may help to elucidate the remission versus cure (Withdrawal of Medication in Recovered DCM (WrecEF), Therapy withdrawal in REcovered Dilated cardiomyopathy trial (TRED)) (217).

We are also pursuing the investigation into the occurrence of recurrent remodeling and the identification of its causal factors and predictors.

And what about HF with mid-range EF (HfmrEF), both HFrEF and HFpEF may evolve to HfmrEF, making unclear whether HFmrEF represents a transitional status between HFpEF and HfrEF or an independent entity on its own. Of notice is the fact that the three HF categories are separated by only a few LVEF percentage points, thus the inter and intra-observer variability of echocardiography may compromise accurate identification of borderline patients (170).

Clearly the subject of reverse remodelling is an area opened to much further research, the result of which holds many promises. Among many others the following areas are of interest:

1- The role of Aetiology.

- LV remodelling and dysfunction are the common phenotypic manifestations of a diverse range of insults, either systemic or heart-specific. Myocardial recovery (or remission) can occur but is more likely to be seen in nonischaemic cardiomyopathies, younger patients, and patients with a more recent onset of the disease. These characteristics overlap with those of patients who are more likely to recover with therapy, including neuro-hormonal blockers, ivabradin and CRT.

2- The place of Advanced Echocardiography:

- To explore the accuracy of LVEF determined by 3D imaging.
- To characterize myocardial substrate using echocardiographic speckle tracking measurements of global longitudinal, radial and circumferential strain, LV torsion and twist.
- To assess regional mechanical forces timing heterogeneity in radial axis and longitudinal dispersion, using strain rate imaging as markers for ventricular arrhythmias.
- To ascertain if persistently abnormal echocardiographic global longitudinal strain can predict the likelihood of the occurrence LVEF decline during follow-up.
- To assess LV contractile reserve by stress echocardiography.

3- The relevance of cardiac MRI:

- In the assessment of the impact of myocardial fibrosis and extracellular matrix quantification (by LGE and T1 mapping, T2 mapping and myocardial extracellular volume fraction) on predicting reverse remodeling.
- In the ability to measure oedema, macroscopic fibrosis and diffuse fibrosis and to quantify the cellular and extracellular compartments is promising. This may allow to personalize therapeutic approaches and to develop new therapies targeted either to interstitial or to intracellular pathways.
- In determining if the evaluation of midwall fibrosis may improve arrhythmia risk stratification and further refine ICD implantation criteria.
- In studying the role of Galectin-3 and others ECM biomarkers in prediction the extent of replacement fibrosis at cardiac MRI.
- In the investigation of the association between LVRR and myocardial fibrosis reversal.

4- The importance of cardiopulmonary stress test:

- In determine the impact of LVRR on exercise capacity when depressed LVEF has increased to 0.50 with pharmacological therapy.

5- The relevance of Myocardial meta-iodobenzylguanidine imaging

- In assessing what manner the localization and quantification of abnormalities in the adrenergic norepinephrine transporter terminals are associated with an increased risk for ventricular arrhythmia and sudden death.

6- The importance of cellular/molecular studies

- To characterize, in transgenic mouse models of reversible dilated cardiomyopathy, transcriptional and epigenetic changes in the myocardium during the failing and recovering phases. New 'reverse remodelling genes' are dysregulated; the function of these latter genes in the reverse-remodelling process is unknown.
- To investigate the changes in mitochondrial structure and function in individuals who have myocardial reverse remodelling and possible recovery.

- To understand the changes in the ECM, incorporating the 3D structure and non-collagen protein changes in addition to the fibrillar component, as a means to study myocardial stiffness and changes in myocardial recovery (74).
- To understand whether miRNA expression patterns can normalize with reverse remodelling is important in the field of myocardial recovery.

7 – The impact of with medical therapies

The effect on reverse remodelling of other experimental therapies for HF, such as neuromodulation (the broad class of therapies include baroreceptor, vagal, spinal-cord stimulation and cardiac contractility modulation), has not been studied; nor have the effects of neprilysin inhibition.

8 – The relevance of studies on mitral percutaneous edge-to-edge repair versus medical therapy on clinical end points and ventricular reverse remodelling.

As percutaneous therapies for functional mitral regurgitation advances, with reduction in the risk of procedural complications and improved efficacy, the benefits of treating functional mitral regurgitation must be further clarified.

9- The importance of studies on effects of LVADs

Different LVADs have different effects on geometrical reverse remodelling: intrathoracic devices tend to push the LV apex towards the base, resulting in a more spherically reverse-remodelled heart, whereas extra thoracic (pre-abdominal) devices pull the LV apex away from the base, resulting in a more elliptically reverse-remodelled heart.

CHAPTER VII

CONCLUSIONS

CONCLUSIONS

LVRR occurred in about one third of our idiopathic DCM patients, especially in those with higher blood pressure values and less advanced disease, who may benefit from maximal drug titration. LVRR was associated with an improvement in NYHA functional class and in prognosis.

In patients with LVRR there was an improvement in diastolic and systolic volumes and in sphericity index. However, despite recovery of LVEF to normal values, frequently there is persistent abnormal circumferential and longitudinal LV strain rate and LV Tei index values, suggesting sustained subclinical left ventricular dysfunction.

CA125, BNP and hsCRP were predictors of clinical severity and congestion. A low baseline value of Galectin-3 may be a predictor of the occurrence LVRR. When analyzing the temporal evolution of biomarker levels of in our population, it became evident that some of them, GDF-15 and MMP-3, continued to rise, despite HF therapy, even in patients with LVRR. We speculate that despite ventricular reverse remodeling, there is persistent matrix fibrosis activation, apoptosis and inflammatory activity. Also, significant correlations were found between the levels of biomarkers and echocardiographic parameters of LV remodeling, supporting the multidirectional pathway of remodeling in HF.

LV mechanical reverse remodeling does not seem to be always associated with LV electrical reverse remodelling and thus arrhythmic risk may persist.

Multiple lines of evidence support the point of view that in most instances, reverse remodeling does not lead to a normal heart, despite reversal of many aspects of the heart failure phenotype. Thus, the regression of the HF phenotype and the accompanying return toward a more normal ventricular shape and function associated with reverse remodeling does not signify that the cellular/molecular biology and physiology of these hearts is normal. This may explain why reverse remodeling may be followed by recurrence of LV dilatation and dysfunction with poor clinical outcomes. This is in favour of maintaining neuro-hormonal blockade and/or device therapy even after LVRR has occurred.

Recognition of this new clinical phenotype, which is coming to be known as a state of HF remission, underscores the need to accurately define and identify reverse modelled myocardium in order to investigate the most appropriated therapies.

CHAPTER VIII

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Left ventricular function recovery in patients with severe longstanding alcoholic dilated cardiomyopathy

Sandra Amorim, MD, J. Silva-Cardoso*, MD, Ph D, Manuel Campelo*, MD, Brenda Moura MD, Elisabete Martins MD, F. Rocha-Gonçalves* MD, Ph D, Cassiano Abreu-Lima* , MD, Ph D
Cardiology Department- Hospital São João, Porto, Portugal

Abstract
Background: In recent and milder forms of alcoholic dilated cardiomyopathy (ADCM) complete recovery of left ventricular dysfunction after abstinence has been reported but this may not be the case in longstanding severe forms.
Aims: To determine the recovery of left ventricular function (LVF) in severe longstanding ADCM after abstinence and to ascertain if it is associated with a better prognosis.
Methods and results: Forty-five consecutive patients with longstanding and severe ADCM aged 44±10 years were followed during 64±4 months. LVF recovery was defined as an absolute increase in left ventricle ejection fraction (LVEF) $\geq$ 0.10 in two consecutive evaluations separated by six months, occurring in the absence of changes in therapy. At the beginning of follow-up, LVEF was 0.26 ±0.8 and 24% of the patients were in NYHA class III or IV. During the follow-up 44% stopped alcohol intake and 36% showed LVF recovery. The baseline characteristics of the patients that stopped alcohol and those that did not were similar. Comparing patients that improved LVF with those that did not the former were more likely to have stopped alcohol (75% *vs* 28%; p<0.01). The former were also more frequently in NYHA class I or II at the end of follow-up (100% *vs* 69%, p=0.02) and had a better survival (100% at 5 years and 80% at 10 years *vs* 64% at 5 years and 32% at 10 years; p<0.01).
Conclusion: Abstinence is an important therapeutic measure even in longstanding severe ADCM and is associated with recovery in LVF, which in turn, is associated with an improvement of symptoms and in prognosis.

Keywords: severe longstanding alcoholic cardiomyopathy, alcohol abstinence, left ventricular function recovery, prognosis

1. Introduction

In the U. S. A. long-term heavy alcohol consumption is the leading cause of non-ischemic, dilated cardiomyopathy (DCM) in both sexes and all races (1). In developed countries it accounts for 3 to 40 % of all cases of DCM and some studies showed that about one third of asymptomatic alcoholics have some evidence of cardiac dysfunction (2).

Although there is no specific daily dose of alcohol consumption or duration of intake that is systematically associated with the development of DCM, patients that have a history of daily consumption ≥ 80-90g for more than five years frequently develop changes in cardiac structure and function. (1,3). Proposed mechanisms responsible for these changes include impaired sarcoplasmic reticulum calcium uptake, inhibition of myosin ATPase, alterations in the fluidity of cytoplasmic membrane and in the function of ion channels traversing the sarcolema (4, 5, 6). These changes ultimately lead to a decrease in myocyte inotropism and a decrease in cardiac output. Initially there is an asymptomatic stage when left ventricular (LV) dilation, increased LV mass, preserved ejection fraction and impaired relaxation are present (7). Later on if the patient continues to drink, the symptomatic stage follows, characterized by pronounced LV

dilation, increased LV mass, LV wall thinning, systolic dysfunction and signs and symptoms of overt heart failure (1).

Some studies showed an association between the presence of point mutations in mitochondrial DNA, or between angiotensin-converting enzyme gene polymorphisms and the development of alcoholic dilated cardiomyopathy (ADCM) (8, 9). Possibly there is interplay between alcohol abuse and genetic susceptibility in the development of ACDM.

Complete recovery of left ventricular function (LVF) after complete abstinence of alcohol intake has been reported but this was observed in early stages of the disease and in a very limited number of patients (10, 11). Therefore, it is not certain if alcohol withdrawal may also be beneficial in end-stage in ACDM patients with heart failure and important structural changes. There is also little information on the impact of alcohol abstinence on the long-term prognosis of patients with ADCM.

The purpose of the present study was to determine if in patients with severe longstanding ACDM alcohol withdrawal may be associated with LVF recovery and to ascertain if this recovery is associated with a better outcome.

2. Methods

2.1 Population

Between January 1988 and December 2002 patients with ACDM attending a Heart Failure Clinic of the Department of Cardiology of a University Hospital were consecutively recruited into this study. We enrolled into this study patients with symptomatic ADCM. DCM was defined according to the World Health Organization criteria (12): LV dilatation with a left ventricle ejection fraction (LVEF) < 0.4. ADCM was defined by a history of heavy alcohol consumption (≥ 80 g/daily for at least 5 years) with other causes of DCM (coronary artery disease, hypertension, valvular disease, hipothyroidism, diabetes or previous treatment with anthracycline,) being excluded (3,12). We defined an enlarged left ventricle by the presence of a LV end-diastolic diameter > 55 mm and/or of a LV end-systolic diameter >37 mm, for a body surface of 1.91 m2 (13). Coronary angiography was performed to exclude ischemic aetiology in case of chest pain in patients with more than two coronary risks factors or with positive stress tests.

In order to better assess the effect of alcohol abstinence the patients had to have a stable LV function at baseline. To ensure this, patients were included in this study only if they had two measurements of left ventricle ejection fraction < 0.40 separated by at least one year. We excluded patients with diabetes, hypertension and with recent episodes of rapid atrial or ventricular arrhythmias.

2.2 Definitions

Total alcoholic abstinence was defined as the complete and sustained withdrawal of alcohol, confirmed by the patient and relatives and by normal levels of γ- glutamyl transferase during follow-up. For the purpose of this study we only considered total abstinence and did not try to evaluate the amount of alcohol intake due to the obvious underestimation of alcohol consumption by the majority of patients.

We considered that a significant improvement in LVF occurred when all the following criteria were satisfied:

1. an absolute increase in LVEF of ≥ 0.10 in two subsequent echocardiograms separated by at least six months;

2. a decrease in LV end diastolic diameter (LVEDD) with no increase in mitral regurgitation, if present;

3. this improvement must had occurred in the absence of changes in medications such as angiotensin-converting enzyme inhibitors (ACEI) or beta-blockers, which are known to have a positive impact in LVF.

Measurements were done by the same observer without knowing the previous exams results. The ejection fraction was calculated by the modified Simpson method (14).

2.3. Data analysis

The results were expressed as mean value ± standard deviation. Variables with a non-normal distribution were expressed as a median value and interquartile range.

The analysis of categorical variables was made by the Qui-square test and Fisher exact test. The analysis of continuous variables was made by the Student's t test or by the Mann-Whitney test. Paired Student's t test was used to compare the values of these variables at the end of follow-up with those at baseline.

The actuarial survival curves were calculated using the Kaplan-Meier method and compared by using the log-rank test.

The SPSS software program (SPSS Inc 10.0; Chicago, Illinois) was used. A p value of <0.05 was adopted for the rejection of the null hypothesis.

3. Results

Fifty-four patients were enrolled in the study. Nine were excluded from analysis because they were lost for follow-up. The patients were followed in our Heart Failure and Transplant Clinic for a maximum of 14 years and a mean of 64±4 months. At baseline their age was 44±10 years. Forty-three (96%) were males, 24% were on NYHA class III or IV and the median of duration of symptoms was 15 months. LVEF was 0.26±0.8, LVEDD was 70.7±6.8 mm and left atrial diameter was 48.6±8.1mm (table 1). The majority of the patients had at least one hospital admission due to decompensated chronic heart failure during the previous year. At admission to the Heart Failure Clinic 78% of the patients were on ACEI, 11% on beta-blockers and 49% on digoxin.

A significant improvement in LVEF according to definition occurred in 16 (35.6%) patients. The mean time to recovery since the beginning of the follow-up was 42±34 months. In these patients LVEF increased from 0.24±0.7 to 0.49±0.8 ($p<0.05$) and this increase in LVF was concomitant with a decrease in left ventricular end-diastolic dimension from 68 ± 6 to 58 ± 7 mm ($p<0.05$).

Although repeatedly advised to do so only 20 patients (44.4%) stopped alcohol consumption. Patients that improved the LVF were more likely to be abstinent (75% *versus* 28%; $p<0.01$) than those who did not (n=29) (table 2). Excluding alcohol abstinence, the improvement in LVF was not associated with other clinical variables such as: age, duration of symptoms, NYHA class, blood pressure or heart rate (table 2). Additionally, the recovery in LVF was not associated with any ECG parameters or with basal echocardiographic dimensions (table 3).

At the end of the follow-up, all patients with an improvement in LVF were in NYHA class I or II versus 69% of the patients without an improvement in LVF (p=0.02) (table 4).

No differences were found between the use of ACEI and beta-blockers among the two groups at the end of follow-up (table 4). The use of digoxin was higher in the group that did not improve LVF due to a worse functional class at the end of follow-up.

Patients showing an improvement in LVEF had an overall survival of 100% at 5 years and 80% at 10 years. Patients that did not improve LVF had an overall survival of 64% at 5

Table1. Basal characteristics of patients with ADCM

	n=45
Male sex %	95.6
Age (years)	44.1±9.7
Duration of symptoms (months)	15.0 ±23.8
NYHA class (III-IV)%	24.4
Systolic blood pressure (mmHg)	122.1±18.4
Heart rate (bpm)	84.3±15.6
ECG	
Atrial fibrillation %	22.7
Left bundle block %	36.4
Echocardiogram	
LV ejection fraction	0.26±0.8
Left atrial diameter(mm)	48.6±8.1
LVEDD (mm)	70.7±6.8
LV mass index (g/m2)	168.6±53.9
Mitral regurgitation grade (III-IV/IV)	24.4
Previous treatment %	
- ACE inhibitors	77.8
- beta- blockers	11.1
- Digoxin	48.9

LV= left ventricle
LVEDD= left ventricular end-diastolic diameter
ACE= angiotensin-converting enzyme

Table 2. Clinical predictors of recovery in LVF.

	No recovery LVF n= 29	Recovery LVF n=16	*p*
Male sex	93.1	100.0	0.53
Age (years)	44.2 ± 9.4	43.9±10.6	0.97
Duration of symptoms (months)	12.9 ± 20.0	7,5 ± 9.9	0.97
Basal NYHA class (III-IV)	27.6	18.8	0.72
Systolic blood pressure (mmHg)	119.8±17.8	126.1±19.2	0.44
Heart rate (bpm)	85.8±16.8	81.7±13.3	0.48
Alcoholic abstinence	27.6	75.0	0.002

Table 3. Laboratorial predictors of recovery in LVF

	No recovery LVF n=29	Recovery LVF n=16	*p*
Echocardiogram			
LV ejection fraction	26.8±8.1	24.1±7.7	0.15
Left atrial diameter (mm)	49.9±7.4	46.1±9.0	0.28
LVEDD (mm)	72.2±6.8	68.2±6.3	0.10
LV mass index (g/m2)	170.4±39.8	165.2±74.3	0.23
Mitral regurgitation grade (III-IV/IV)	27.6	18.8	0.72
ECG			
Atrial fibrillation	27.6	13.3	0.45
Left bundle branch block	44.8	20.0	0.10
Na serum (mEq/l)	139.1±4.6	140.6±3.4	0.26

LV= left ventricle
LVEDD= left ventricular end-diastolic diameter

Table 4.Functional status and therapy at the end of follow-up

%	Total n=45	No recovery LVF n= 29	Recovery LVF n=16	*p*
NYHA class I-II	79.5	69.0	100.0	0.02
ACEI / ARB	100.0	100.0	100.0	1.00
beta- blockers	44.4	41.4	50.0	0.58
Digoxin	55.6	72.4	25.0	0.002

ACEI= angiotensin-converting enzyme inhibitors
ARB= angiotensin II receptor antagonists

Figure1. Survival curves of death or heart transplantation in patients with and without recovery in LVF

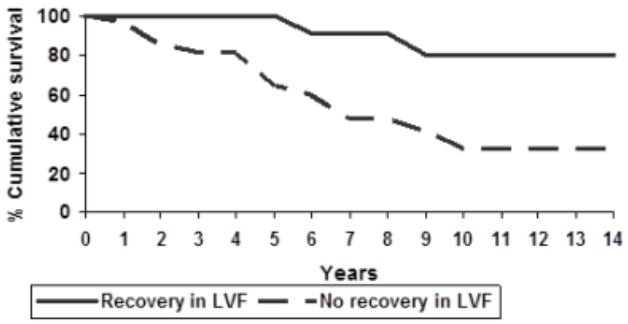

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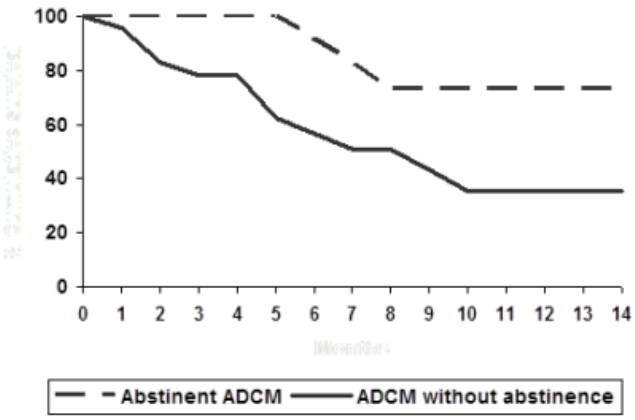
Figure 2.Survival curves of death or heart transplant in abstinent and non-abstinent ADCM patients



years and 32% at 10 years ($p<0.01$) (figure 1). Patients with ADCM and abstinence had a significantly better outcome (100% survival at 1, 5 years and 73.2% at 10 years) than those who continued to drink (95.9% survival at 1 year, 62.3% at 5 years and 35.5% at 10 years) ($p=0.02$) (figure 2).

4. Discussion

In our patients with severe longstanding ADCM, abstinence was an important therapeutic measure being associated with a significant improvement in LVF in a relatively high percentage of them.

There are reports from experimental (15) and clinical studies (10, 11, 16) that alcohol abstinence may improve LVF at least in early stages of DCM. However, longstanding ADCM is generally viewed as an irreversible disease (17, 18). Our is the first study to show that even in severe cases of longstanding ADCM, alcohol abstinence may be associated with improvement in left ventricular dimensions and function and improvement in heart failure symptoms and prognosis.

Nicolàs and coworkers (2) reported that patients who completely abstained from alcoholic beverages or reduced alcohol intake by 40%, had a significant improvement in LVF by 0.13 and 0.12, respectively. However, left ventricular systolic function compromise was only moderate (LVEF=0.39 on average) in this patient sample. Below 60 g/day of alcohol consumption no damage to the myocardium was found in this study.

Guillo P et al (19) studied a small group of patients (n=14) with severe DCM (mean EF= 15). Only nine were followed during 36 months. They observed a LVF recovery accompanied by functional capacity improvement and an excellent outcome in six patients who stopped drinking.

The main relevance of our study lies in the fact that in our group of 45 patients the symptoms of heart failure were present for an average of 15 months and there was a markedly reduced LVEF and a significant LV dilatation. Additionally, two measurements of LVEF <0.40 separated by at least one year had to be obtained for inclusion in the study group, so

patients with recent DCM or subclinical myocarditis were excluded. We also excluded patients with diabetes and hypertension, because these risk factors might be related to development of cardiomyopathy. Patients with recent episodes of rapid atrial or ventricular arrhythmias were also excluded because of the well know relation of these arrhythmias to transient LV systolic dysfunction. In contrast with previous investigations, our study used very stringent criteria of left ventricular function recovery in patients with ADCM and was the first to evaluate its predictors.

There was no statistically significant difference regarding therapy among the patients that recovered LVF as compared with those that did not. It is note worthy that the level of prescription of beta-blockers (44%) was significantly lower that the 78% reported in the general population of our Heart Failure clinic (20). This was due to a low compliance shown by this group of patients regarding this particular form of therapy.

As previously mentioned, we did not attempt to quantify the daily alcohol intake due to the lack of precision of such estimates. Besides, the threshold of alcohol consumption for developing cardiomyopathy varies widely between individuals and there are large variations in the literature (7, 17, 21).

Our patients that showed a recovery in LVF had a better prognosis and showed an improvement in symptoms. Those who stopped alcohol consumption had a better outcome than those that did not.

There is controversy regarding the effect of alcohol abstention on prognosis and symptoms of heart failure in patients with ADCM (2, 18, 19, 21).

Fauchier (3) showed that lack of abstinence from alcohol after the diagnosis of ADCM was a strong and independent predictor of cardiac death. However he found that patients with ADCM that stop alcohol intake do not have a better prognosis than patients with idiopathic dilated cardiomyopathy (IDCM).

On the contrary Prazak (22) showed that survival of patients with ADCM was significantly better than patients with IDCM. He associated this with the fact that patients with ADCM received a more aggressive medical treatment and a firm advice to refrain from alcohol consumption and to enrol in programs of alcohol withdrawal.

Gavazzi coordinated a Multicenter registry (21) that analysed the long term outcome of 379 males with IDCM according to alcohol consumption, excluding patients with end- stage disease. The seven-year transplant-free survival was significantly lower in alcohol abusers than in patients with IDCM, and significantly lower in patients who continued alcohol intake than in patients with IDCM or patients who stopped alcohol intake. Only patients who abstained from alcohol consumption had an improvement in LVEF.

Balk in an editorial comment (4) considered that ADCM patients do not became totally abstinent. By combining the results of Faucher's and Prazak's studies he gives a negative answer to the question posed in his editorial title:"are a few drinks allowed in dilated cardiomyopathy?". This is in contrast with Nicolàs's (2) findings that moderate drinking was associated with an improvement in LVF and in short-term survival, in a similar way as patients who totally abstained from alcohol.

Our study, which describes an objective left ventricular function improvement, emphasizes the role of alcohol in left ventricular dysfunction. Not all patients with chronic alcoholism develop cardiac dysfunction. ADCM is

possibly a multifactorial disease where environmental and genetic factors influence the pathogenesis and the course of the disease.

Limitations of the study

In our study we didn't compare ADCM with IDCM patients in terms of their alcohol consumption behaviour. This is a subject of another study which we are pursuing.

We didn't take into account the prevalence of ventricular arrhythmias, recognized as prognostics parameters of heart failure. We also did not perform non-invasive tests for corroborating the reversal of myocardial damage, as the use of myocardial anti-myosin antibody uptake (23) nor the use of haemodynamic or morphometric analysis by cardiac catheterization (24).

5. Conclusion

In patients with severe longstanding ADCM prognosis is good if complete abstinence is accomplished and it is associated with an improvement in LVF. Our work also underscores the role of alcohol in the pathogenesis of ADCM.

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